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The primary mission of the Military Molecular Medicine Initiative (MMMI), a congressionally-supported military-civilian collaboration between WRMC, Windber Medical Center (WMC) /Windber Research Institute (WRI) is to: 1) Teach, implement and study lifestyle changes added to the "best" medical practices that promote cardiovascular health; 2) Identify patients at risk earlier by characterziaing cardiovascular disease at the molecular disease stage and identify biomarkers predictive of sub-clinical CVD; and 3) Relate genomic/proteomic changes to the evolution of CVD risk factors in response to lifestyle changes in an effort to prevent, arrest or reverse CVD.

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Introduction

The epidemics of cardiovascular disease (CVD), Type II diabetes, and obesity generate a major share of the preventable costs of American health care. Currently, the American health care market place does not support preventive care that would save lives and costs associated with these problems. Healthcare costs are predicted to rise from 16% of the US GDP in 2005 to 30% of the GDP by 2025 if we fail to invest in prevention. Molecular biology is providing new information on key processes that have the potential to greatly improve preventive cardiac and metabolic health. The primary mission of the ***Military Molecular Medicine Initiative (MMMI),*** a congressionally-supported military-civilian collaboration between Walter Reed Army Medical Center (WRAMC) and Windber Medical Center (WMC)/Windber Research Institute (WRI) is to: 1) Teach, implement and study lifestyle changes added to "best" medical practices that promote cardiovascular health; 2) Identify patients at risk earlier by characterizing CVD at the "molecular" disease stage and identifying biomarkers predictive of subclinical CVD; and 3) Relate genomic/proteomic changes to the evolution of CVD risk factors in response to lifestyle changes in an effort to prevent, arrest or reverse CVD. Within these objectives, the MMMI will include: a) a comprehensive and innovative CVD risk factor assessment in the military beneficiary population; b) advanced imaging methods for quantifying numerous aspects of heart health in military and other populations; c) an optimal healing environment for CVD patients; and d) an integrated statistical analysis of clinical and molecular data to identify patterns of CVD risk factors that will allow a unique and intensive collection of data at the clinical and molecular levels for heart disease, but with applicability and relevance in patients with other chronic diseases such as cancer, diabetes, metabolic syndrome and obesity. The heart disease data base will provide the ability to find novel disease markers, new treatment approaches, and provide a unique venue for future research.

Body

Task #1: Complete data analysis of "Non-Invasive Coronary Artery Disease Reversal" (CADRe) Study Protocol conducted at WRAMC.

Status: Final cohort of study participants completed the study intervention in April 2005. Major data analysis has been completed with development of manuscripts in progress. Planned publications are as follows:

Background: Over 25 years ago, Dean Ornish began pioneering efforts to demonstrate that lifestyle change (ultra-low fat vegan diet; yoga as a stress management technique; aerobic exercise; group support) could be an effective alternative to medications and revascularization procedures for CHD patients. Ornish's initial 30-day study of 10 CHD patients, who participated in the diet and stress management interventions within a residential setting[1], demonstrated increased treadmill time as well as reduced anginal episodes, blood pressure, premature ventricular contractions and lipids. These findings were confirmed in a similar 24-day study that compared CHD patients in the lifestyle intervention (n=23) with those receiving customary care (n=23): 44% increase in treadmill time; reductions in angina (91%), total cholesterol (21%), triglycerides (15%) and HDL cholesterol (17%).[2] The Lifestyle Heart Trial[3] added an aerobic exercise component and was designed to assess long term maintenance of the comprehensive lifestyle program outside a residential setting along with its impact on myocardial perfusion and angiographically-determined progression of CHD. Although randomization assignment was known by subjects prior to consent, there was an intervention (n=28) and control (n=20) group in this study. At one year, 82% of the intervention group (n=22) had a measurable regression of

lesion severity, contrasted by lesion progression in 53% of controls (n=19). The intervention group had a 91% decrease in anginal frequency compared to a 165% increase in the control group. Positron emission computed tomography (PET) scans showed improved myocardial perfusion in the intervention group, as opposed to a significant progressive worsening in the controls.[4] At five years, statistically significant improvements in the intervention (n=20) versus control (n=15) group were lower weight, smaller coronary lesion diameter, and fewer cardiac events, although the impact of the intervention on weight and lipids was diminished compared to results at one year.[5] The Ornish Program subsequently has been implemented in several US sites as the Multi-center Lifestyle Demonstration Project with three- month and one-year results in 342 CHD patients showing improvements in several CV risk factors.[6]

Hypothesis: An intensive, multi-component, lifestyle intervention will favorably impact CVD risk factors and CHD symptoms in military health care and rural community populations.

Study Design/Purpose: This prospective, non-randomized, single-arm (treatment), observational trial, in which each individual serves as his/her own control, comparing outcomes to baseline data was conducted to determine if comprehensive lifestyle changes (low-fat vegan diet supplemented with soy, moderate aerobic exercise, stress management, and group support) can slow, stop or reverse the progress of coronary artery disease. Several efficacy and feasibility outcomes were assessed in this study.

Efficacy
Anginal frequency and New York Heart Association (NYHA) class
Carotid plaque thickness change at 1-year (Since April 2001)
Cardiovascular fitness, measured in metabolic equivalents (METS)
Standard biochemical markers (blood lipids, glucose)
Emerging biochemical markers (C-reactive protein, fibrinogen, homocysteine)
Body composition (weight, BMI, % body fat) and blood pressure
Psychosocial profiles (hostility, depression, stress, perceived support, quality of life)

Feasibility
Study completion rate
Adherence rates- overall and for individual study components

Subject Enrollment and Demographics
Enrollment goal of 200 subjects was reached in April 2004. A total of 714 subjects were screened to enroll the 13 study cohorts between February 2000 and March 2004. The final cohort of subjects completed the 1-year study in April 2005. Fifty-six subjects (28%) withdrew from the study, the majority within the first 3 months. See Figure 1 for study subject distribution. Of those subjects who withdrew, the majority of subjects (64%) withdrew from the study for lack of commitment to the program; 27% became ineligible during the screening process, and; 9% withdrew due to military separation or reassignment. A high dropout rate was anticipated with this study.

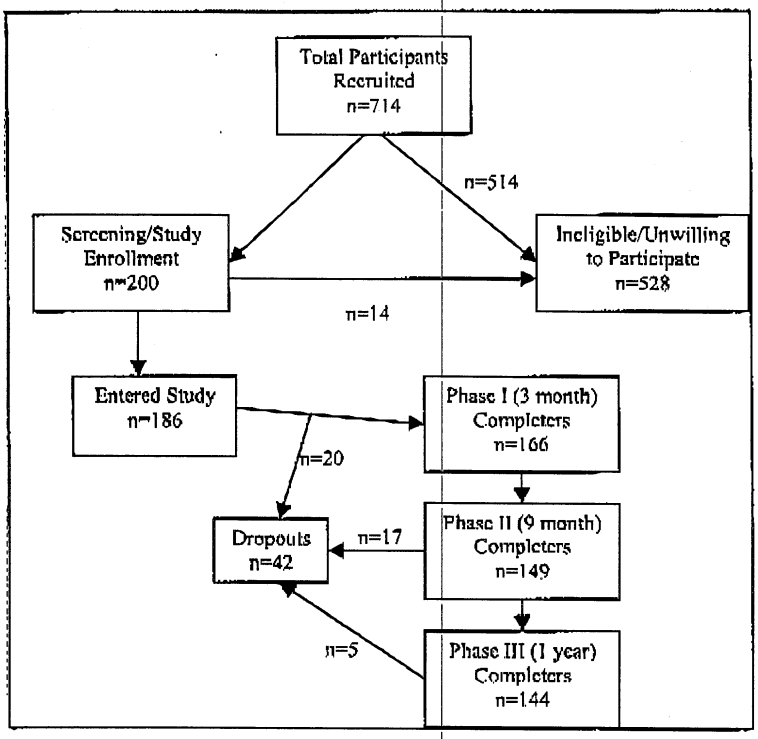


Figure 1. Study Subject Disposition

Of those 144 subjects completing the year long study, the mean age was 60.6 ± 9.7 with a range of 36 to 80 years old, 29% were female, and 16% were from minority groups. Sixty-eight percent had documented coronary artery disease (CAD). Of those with CAD, 82% had at least one revascularization procedure (bypass surgery or angioplasty). Additionally, 67% of the subjects suffer from hypertension, 18% were diabetics and 76% were taking cholesterol-lowering medications at enrollment. Of the subjects who completed the study, 15% were active duty, 63% were retired from the military services, and 22% eligible family members. Differences between demographic and baseline characteristics of all enrolled subjects as compared to study completers is displayed in Table 1. At baseline, study completers were overweight (BMI = 29.8 ± 5.8; weight =198 ± 48 lbs) with 48% of subjects considered obese (BMI > 30). Subject fitness, as measured by peak MET level achieved on maximal treadmill testing (9.5 ± 209) was average for this age group.[7] As compared with the average US diet, the typical subject's baseline diet was characterized as low fat (23.9% of total calories).[8]

Table 1. Demographics and Baseline Characteristics: Enrolled vs. 1-Year Completer Subjects

	Enrolled (n=200)	1-Year Completers (n=144)	P
Age (years)	59.7 ± 9.9	60.6 ± 9.7	0.04
Male (%)	70.5	71.5	0.61
Caucasian (%)	80.0	84.0	0.04
BMI (kg/m^2)	29.8 ± 5.9	29.8 ± 5.8	0.89
Fitness (MET Level)	9.4 ± 2.9 (n=187)	9.5 ± 2.9 (n=137)	0.67
Dietary Fat (%)	25.0 ± 9.6 (n=162)	24.1 ± 9.4 n=129)	0.01
CAD (%)	63.5	68.1	0.04
HTN (%)	66.5	67.4	0.74
Diabetes (%)	20.0	18.1	0.32
Hyperlipidemia (%)	93.0	95.8	0.02

*n=187; n=137

Subject Adherence:

The study intervention consisted of an ultra low fat diet (<10% total calories as fat; 5-10 mg cholesterol/d, soy and legumes as the protein source, limited nonfat dairy products, 35-50 grams of fiber, and >5 servings of fruit and vegetables daily), moderate aerobic exercise (180 min/wk), group support, and stress management practice (60 min/day). Attendance at group support was not measured for this study. Participants provided dietary food pattern, structured exercise activity and stress management practice activity data daily via a Health Questionnaire at baseline and a weekly adherence log over the course of the study. Exercise and stress management activity time data for the 3-month follow-up time was calculated using the mean scores of study weeks 8-12; 1-year data was calculated using the mean score of study weeks 39-52. Total fat grams and cholesterol (mg) data was obtained through periodic 3-day food records analyzed with Nutritionist V software (Version 2.2; First DataBank, San Bruno, CA) at baseline, 3 months (closest to week 12) and 1-year (closest to month 12).

Data throughout the study has shown that study subjects are able to make and maintain comprehensive changes in nutrition and lifestyle both in the short and long term (Table 2). Subjects were able to achieve significant changes in diet, exercise and stress management goals by 3 months and largely sustained at one year.

Table 2. Study Intervention Adherence at 3 Months and 1-Year

	3 Month (n=166)	1-Year (n=144)
Exercise		
Min/wk	200.0 ± 80.8	170.6 ± 80.3
% Study Goal	111.1 ± 44.9	94.8 ± 44.6
Diet*		
% Study Goal	91.9 ± 9.8	88.5 ± 15.3
Stress Management		
Min/wk	294.2 ± 112.6	243.0 ± 155.4
% Study Goal	70.0 ± 26.8	57.9 ± 37.0
Overall Adherence (%)*	91.1 ± 22.2	81.3 ± 24.2

Values are mean ± SD. Other than diet, % adherence is not capped at 100%.
***3 month N=165; 1-year N=134.**

Outcome Data:

The number of participants enrolled in this study with a history of angina has been extremely limited. As a result, meaningful data analysis of angina frequency as the primary endpoint will not be performed.

Measurement of carotid intimal media thickness (CIMT) at baseline and 1-year as a surrogate marker for subclinical atherosclerosis was conducted on subjects beginning April 2001. Baseline and 1-yr data were analyzed on 60 subjects (mean age: 58.5 ± 9.5 years). Data analysis, using the Wilcoxon Signed Ranks Test, showed no significant changes in the mean thickness (0.731 vs. 0.720; p=0.324). However, a sub analysis was performed to determine the overall change in CIMT and the relationship between CIMT change and number of healthy lifestyle measures achieved (range 0 – 5) in a 5-component Heart Health Index (HHI): BMI < 25 kg/m^2, exercise ≥ 150 min/wk, BP<140/90 mmHg, LDL Cholesterol (LDL-C) < 100 mg/dL, fiber intake > 25 g/day. The change (-0.011 ± 0.118 mm) between CIMT at baseline (0.731 ± 0.151 mm) and 1-yr (0.720 ± 0.129 mm) was not statistically

significant (p= .48, paired t-test) (Figure 2). From comparable baseline values, CIMT progression differed significantly between participants with a 1-year HHI Score ≥3 (n=43) and those with a HHI Score < 3 (n=17): CIMT change -0.030 ± 0.114mm vs. +0.038 ± 0.118mm; p=0.04 by one-way ANOVA (Figure 3).

Figure 2. CIMT Mean Change (Baseline vs. 1-Yr)

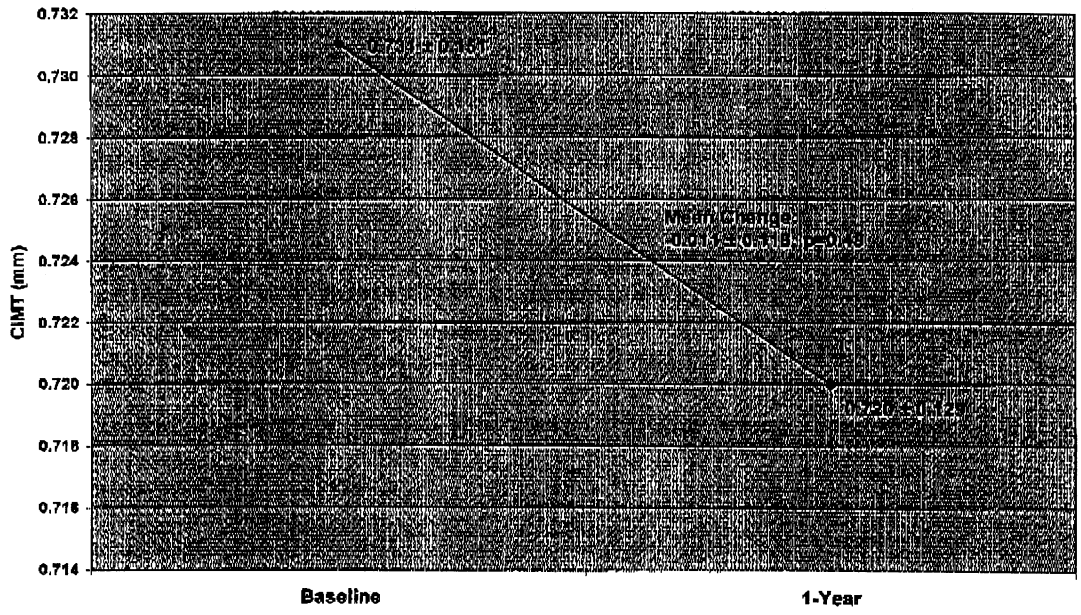
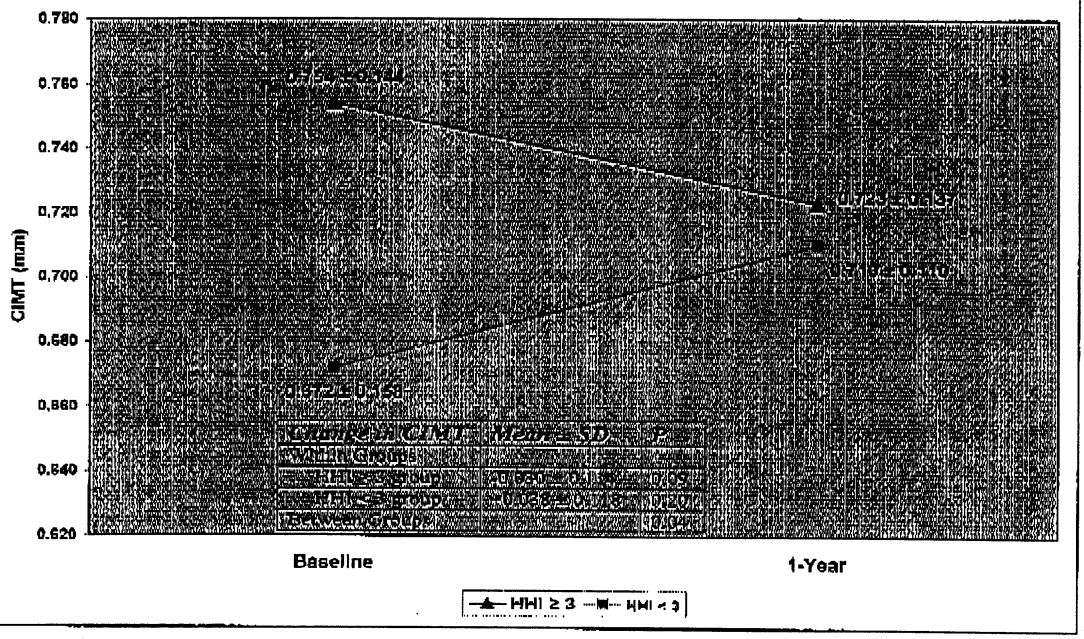


Figure 3. Comparison of CIMT Mean Change by HHI Group



Modest heart healthy lifestyle changes, particularly a reduction in daily consumption of dietary fat, an increase in dietary fiber and daily physical activity, have been shown to significantly impact body composition, blood pressure and serum lipids and certain inflammatory markers. Study subjects who completed the initial 3-months (Phase 1) of the study (n=166) were able to achieve significant improvement in levels of virtually all of the measured CV risk factors (Table 3A). Data analysis, comparing 3 month to baseline, using the paired *t-test* or Wilcoxon Signed Ranks Test was conducted on all major outcome variables. Subjects had an average weight loss 14 lbs. (7%) with 9% loss in body fat. Physical fitness increased 21% after 3 months of structured activity (200 ± 81 minutes/week) and systolic and diastolic blood pressure were improved to a normotensive range. Although 72% of the 3-month completers were on stable statin therapy, subjects received additional benefits from lifestyle change and conferred significant reductions in total cholesterol (13%) and LDL-cholesterol (16%). Despite significant dietary changes (decreased fat, increased fiber) and increased physical activity, a 12% reduction in HDL-cholesterol (49.3 ± 15.9 vs. 42.2 ± 10.7, p<0.0001) was noted from baseline. Triglycerides remained unchanged. No significant changes were seen in C - reactive protein (CRP) or lipoprotein-a, whereas, fibrinogen levels increased 5% and homocysteine levels fell by 2%.

Table 3A. Major Outcome Variables in Phase I – Study Completers

	Baseline	3 Months	Change	P
Body Composition & Fitness				
Weight (lbs) (n=165)	199.9 ± 50.4	186.2 ± 45.1	-13.7 ± 9.8	<0.0001
BMI (kg/m^2) (n=165)	30.0 ± 6.1	28.0 ± 5.4	-2.0 ± 1.4	<0.0001
% Body Fat (n=162)	28.1 ± 8.1	25.6 ± 7.9	-2.5 ± 3.0	<0.0001
MET Level (n=154)	9.5 ± 2.9	11.0 ± 3.0	1.6 ± 1.7	<0.0001
Blood Pressure (n=166)				
Systolic (mmHg)	128.5 ± 16.3	120.7 ± 16.0	-7.8 ± 16.4	<0.0001
Diastolic (mmHg)	74.1 ± 9.7	67.8 ± 9.0	-6.2 ± 11.0	<0.0001
Lipids (mg/dL) (n=166)				
Total Cholesterol	179.8 ± 41.1	153.3 ± 37.1	-26.5 ± 31.9	<0.0001
LDL-Cholesterol	104.8 ± 30.6	86.4 ± 25.8	-18.5 ± 22.0	<0.0001
HDL-Cholesterol	49.3 ± 15.9	42.2 ± 10.7	-7.0 ± 9.8	<0.0001
Triglycerides	156.8 ± 93.7	166.1 ± 103.3	9.2 ± 91.1	0.193
TC/HDL	3.9 ± 1.1	3.8 ± 1.1	-0.1 ± 0.8	0.127
Inflammatory Markers (n=166)				
C-Reactive Protein (mg/L)	3.3 ± 4.2	2.9 ± 3.5	-0.4 ± 3.9	0.184
Homocysteine (umol/dL)	9.3 ± 2.7	8.8 ± 2.6	-0.5 ± 2.2	0.006
Fibrinogen (mg/dL)	383.6 ± 77.9	397.4 ±85.8	13.8 ± 67.4	0.009
Lp (a) (mg/dL)	41.2 ± 46.6	42.8 ± 50.5	1.6 ± 19.9	0.297
Nutritional Values (n=146)				
Total Kcal	1705.0 ± 560.1	1699.0 ± 407.8	6.0 ± 569.1	0.899
% Fat	24.6 ± 9.5	8.8 ± 2.5	-15.7 ± 9.4	<0.0001
% Carbohydrate	56.0 ± 11.5	73.3 ± 4.8	17.3 ± 11.1	<0.0001
% Protein	17.6 ± 4.2	16.4 ± 3.1	-1.2 ± 4.5	0.002
Fiber (g/day)	24.0 ± 12.8	49.0 ± 17.8	25.0 ± 17.1	<0.0001

Values are mean ± SD.

Results of the major outcomes variables for baseline, 3 months and 1-year in study completers (n=144) are presented in Table 3B. Data analysis, comparing 3 month and 1-year to baseline, using the paired *t-test* or Wilcoxon Signed Ranks Test was again conducted on all major outcome variables. Measures of obesity including weight and BMI declined 6%, percent of body fat was reduced by 9%, levels of total cholesterol were reduced by 14% and LDL-cholesterol decreased 17%, systolic and diastolic blood pressure dropped 6% and 7% respectively and measures of physical fitness increased by 22%. Dietary fat was also dramatically lower by 57% and dietary fiber increased by 131%. Despite these positive changes, a 16% increase in serum triglycerides and 13% reduction in HDL-cholesterol were seen although not statistically significant. A 5% increased in serum fibrinogen and a 3% reduction in homocysteine were noted at 3 months, however, no significant changes were seen in levels of CRP or lipoprotein-a.

At 1-year, subjects were able to maintain weight and BMI reduction and percent body fat continued to improve to 10%. Physical fitness levels continued to improve to 24% from baseline assessment. Systolic and diastolic blood pressure levels increased slightly, but still lower than upon study entry. Levels of serum total cholesterol and LDL-cholesterol regressed slightly to 5% and 8%, but still remained lower pre-intervention levels. Triglycerides returned to baseline levels and the HDL-cholesterol depression seen at 3 months improved by 1-year (-7.3 ± 10.1 vs. -1.9 ± 7.5, p=0.002). Although lipoprotein-a was stable over the course of the study, significant improvements were seen in CRP, homocysteine and fibrinogen.

Subjects with documented CAD improved their assessed New York Heart Association (NYHA) functional class as a result of active participation in a regular and monitored exercise program. The NYHA functional class was analyzed using the Wilcoxon Signed Ranks Test. Seventy-four percent of CHD study completers were assessed as having a NYHA Class I (no physical limitations) with the remaining 26% assessed as either Class II or III (slight to marked physical limitations) at baseline. When comparing NYHA functional class at baseline to 12 months, 80% maintained their functional class and 17% improved by 1 grade at 1-yr (p=0.003).

Given improvements in CV fitness and NYHA functional class, positive changes in overall physical health as measured by serial SF-36 Physical Component Scores (PCS) could be anticipated. The SF-36 PCS was analyzed using paired t-tests comparing 3-month and 1-yr to baseline in both Phase 1 completers and study completers (See Table 4A & 4B). In Phase 1 completers (n=165) who completed the SF-36, scores improved from 45.2 ± 9.1 vs. 47.7 ± 9.4, p<0.0001. Analysis of all study completers (n=140) showed continued improvement over the course of the study from baseline assessments.

Table 3B. Major Outcome Variables in Study Completers

	Baseline	3 Months	Change from Baseline	P	1-Year	Change from Baseline	P
Body Composition & Fitness							
Weight (lbs) (n=142)	198.2 ± 48.2	184.9 ± 43.2	-13.3 ± 9.7	<0.0001	186.5 ± 48.1	-11.7 ± 16.7	<0.0001
BMI (kg/m^2) (n=142)	29.8 ± 5.8	27.9 ± 5.1	-2.0 ± 1.4	<0.0001	28.0 ± 5.7	-1.8 ± 2.5	<0.0001
% Body Fat (n=139)	27.9 ± 7.9	25.3 ± 7.3	-2.6 ± 2.8	<0.0001	25.0 ± 7.5	-2.9 ± 3.9	<0.0001
MET Level (n=130)	9.5 ± 2.9	11.3 ± 3.0	1.7 ± 1.7	<0.0001	11.5 ± 3.4	2.0 ± 2.2	<0.0001
Blood Pressure (n=142)							
Systolic (mmHg)	129.4 ± 16.7	120.6 ± 16.4	-8.8 ± 16.7	<0.0001	121.7 ± 14.6	-7.7 ± 17.0	<0.0001
Diastolic (mmHg)	74.1 ± 9.8	67.9 ± 9.2	-6.2 ± 11.2	<0.0001	70.4 ± 9.1	-3.7 ± 10.0	<0.0001
Lipids (mg/dL) (n=144)							
Total Cholesterol	179.5 ± 40.4	151.7 ± 37.2	-27.8 ± 33.2	<0.0001	168.9 ± 40.2	-10.6 ± 31.9	<0.0001
LDL-Cholesterol	104.5 ± 29.7	85.1 ± 25.3	-19.3 ± 22.4	<0.0001	94.4 ± 28.3	-10.1 ± 22.6	<0.0001
HDL-Cholesterol	49.2 ± 15.5	41.8 ± 9.9	-7.3 ± 10.1	<0.0001	47.2 ± 13.1	-1.9 ± 7.5	0.002
Triglycerides*	161.5 ± 96.1	169.1 ± 106.2	7.6 ± 94.1	0.150	166.8 ± 91.2	5.3 ± 77.1	0.106
TC/HDL	3.8 ± 1.0	3.7 ± 1.1	-0.1 ± 0.8	0.152	3.7 ± 1.0	-0.1 ± 0.8	0.069
Inflammatory Markers							
C-Reactive Protein (mg/L) (n=144)*	3.2 ± 4.0	2.9 ± 3.4	-0.4 ± 3.6	0.057	2.4 ± 2.5	-0.9 ± 3.1	0.0004
Homocysteine (umol/dL) (n=144)	9.4 ± 2.7	8.9 ± 2.6	-0.5 ± 2.2	0.004	8.4 ± 2.9	-1.0 ± 2.4	<0.0001
Fibrinogen (mg/dL) (n=143)	385.5 ± 79.5	400.5 ± 89.0	15.0 ± 67.9	0.009	370.5 ± 74.4	-15.0 ± 59.9	0.003
Lp (a) (mg/dL) (n=143)	41.5 ± 47.9	43.0 ± 52.1	1.4 ± 20.6	0.405	42.3 ± 46.7	0.7 ± 25.2	0.733
Nutritional Values (n=101)							
Total Kcal	1821.0 ± 573.8	1778.6 ± 388.1	-42.5 ± 587.5	0.469	1753.6 ± 410.1	-67.5 ± 559.5	0.228
% Fat	23.9 ± 9.1	8.9 ± 2.5	-15.0 ± 8.8	<0.0001	9.8 ± 3.0	-14.1 ± 9.0	<0.0001
% Carbohydrate	57.0 ± 11.1	73.4 ± 5.0	16.5 ± 10.1	<0.0001	72.5 ± 4.9	15.6 ± 10.6	<0.0001
% Protein	17.3 ± 3.7	16.1 ± 2.9	-1.2 ± 4.0	<0.004	16.0 ± 2.4	-1.3 ± 3.8	<0.001
Fiber (g/day)	26.3 ± 13.2	51.6 ± 16.6	25.3 ± 15.9	<0.0001	47.9 ± 15.5	21.6 ± 16.1	<0.0001

*Wilcoxon-Signed Rank Test; Values are mean ± SD.

Psychological assessments of depression, hostility, stress and mental health functional status were collected on study subjects at baseline, 3 month and 1-yr. Results of paired *t-tests*, comparing 3-month and 1-year to baseline assessments, are outlined in Table 4A & 4B, for both Phase 1 and 1-yr study completers. The Center for Epidemiological Studies Depression Scale (CES-D) scores ≥16 are considered positive for the presence of depressive symptoms and scores ≥23 suggest the presence of clinical depression. Enrolled subjects had a mean baseline CES-D score of 8.0 ± 7.3. In Phase 1 completers, 89% had CES-D<16 and ≤8 in 74% of those participants. In study completers, CES-D scores significantly improved (P<0.0001) over the 1-yr.

A hostility score, as measured by the modified Cook-Medley Hostility Scale (CMHS), of 16.5 in women and 18 in men are considered "red" flag scores. Baseline scores of enrolled subjects were low (7.8 ± 4.7) with 188 participants having scores of 16.5 or below with over 70% of those participants with scores ≤9. In study completers, hostility scores significantly improved over the course of the study (P<0.0001) with improvements at both 3 months and 1-year.

Perceived Stress Scale (PSS) scores ≥18 are considered high and would trigger a clinical action. Fifty-nine percent of enrolled subjects had a PSS score ≤12 with a mean score of 11.6 ± 7.2. Significant improvements in PSS scores were seen in Phase 1 completers (p<0.0001) and study completers at 3 months (p=0.001) and 1-yr (p<0.0001).

Overall improvements seen by study participants in the above psychological assessments should manifest overall improvement in mental health as measured by serial SF-36 Mental Component Scores (MCS). A score of ≤41 is considered severely impaired. Enrolled subjects reported a baseline mean MCS score of 52.2 ± 9.8. Phase 1 completers showed a +1.7 ± 8.7 (p=0.013) improvement in functional mental health. Study completers also showed a significant improvement (+2.4 ± 9.1, p=0.003) from baseline to 1-year.

Table 4A. Psychological Survey Scores in Phase I – Study Completers

	Baseline	3 Months	Change	P
Center for Epidemiological Studies-Depression Scale (CES-D) (n=165)	7.5 ± 6.8	6.4 ± 7.3	-1.2 ± 6.1	0.017
Modified Cook Medley Hostility Scale (CMHS) (n=165)	7.5 ± 4.6	6.8 ± 4.8	-0.8 ± 3.3	0.005
Perceived Stress Scale (PSS) (n=164)	11.5 ± 6.8	9.4 ± 6.2	-2.1 ± 6.2	<0.0001
SF36 Physical Composite Score (PCS) (n=164)	45.2 ± 9.1	47.7 ± 9.4	2.5 ± 7.6	<0.0001
SF36 Mental Composite Score (MCS) (n=164)	53.0 ± 9.2	54.8 ± 8.5	1.7 ± 8.7	0.013

Values are mean ± SD.

Table 4B. Psychological Survey Scores in Study Completers

	Baseline	3 Months	Change from baseline	P	1-Year	Change from baseline	P
CES-D (n=141)	7.6 ± 7.0	6.3 ± 7.5	-1.3 ± 5.8	0.009	5.2 ± 5.9	-2.4 ± 6.3	<0.0001
CMHS (n=141)	7.4 ± 4.6	6.9 ± 4.9	-0.5 ± 3.1	0.054	6.1 ± 4.6	-1.3 ± 3.6	<0.0001
PSS (n=139)	11.5 ± 6.9	9.8 ± 6.4	-1.7 ± 6.1	0.001	8.4 ± 5.5	-3.1 ± 6.4	<0.0001
SF36 PCS (n=140)	45.3 ± 9.3	47.8 ± 9.3	2.5 ± 7.2	<0.0001	48.3 ± 9.9	3.1 ± 7.9	<0.0001
SF36 MCS (n=140)	52.9 ± 9.3	54.1 ± 8.9	1.2 ± 8.6	0.095	55.3 ± 7.8	2.4 ± 9.1	0.003

Values are mean ± SD.

Adverse Events

All adverse events are submitted and adjudicated by the WRAMC HUC and USUHS Office of Research after review by both the Principal Investigator and Medical Monitor. Over the course of the study, 135 adverse events occurred over the course of the study including one death as a result of a massive right hemisphere hemorrhagic CVA. This death was not attributed to the study intervention. There were 4 adverse events that were a direct result of the study and potentially life-saving (i.e. screening cardiac stress testing resulting in a revascularization procedures) and 2 that were potentially related to the study. Table 2 below summarizes the adverse events as either cardiac or non-cardiac.

Table 5. Summary of Study Adverse Events

Adverse Event	# of Episodes
Death	1
Cardiac related hospitalizations / ER visits / Cardiac catheterizations with or without interventions	69
Non-cardiac related hospitalizations/ER visits	65

CONCLUSIONS

Our findings show that in a population with stable coronary heart disease or coronary risk factors, participation in an Ornish lifestyle modification program results in substantial improvements of body composition, blood pressure, and fitness as well as total and LDL cholesterol. Some of these changes rival what is observed with pharmacological treatment. Data analysis has confirmed the Ornish Program's overall efficacy for coronary risk factor reduction and inflammation in participants who are adherent. However, recruitment and retention was difficult: only 28% of individuals screened (n=714) subsequently enrolled in the study and 28% of these highly motivated enrollees dropout of the study, the majority within the first three months.

Our results demonstrating the impressive physiologic and psychosocial impact of this lifestyle intervention may translate into favorable long-term outcomes for our study participants. Although analysis of the available CIMT data does not seem to suggest a significant change in measurable atherosclerosis of a small subset of participants, changes may exist when this data is further explored with number of achieved healthy lifestyle measures as covariates. Participation in an intensive lifestyle intervention program with achievement of CV health measures appears to promote carotid atherosclerosis regression while less successful participation results in atherosclerosis progression. This information may be useful in encouraging individuals to adopt healthy lifestyle changes.

There is a consistent finding of HDL depression. Marshall et al[9] relate an ultralow-fat diet as a component of this lifestyle intervention induced reductions in HDL-cholesterol and the emergence of a dyslipidemic lipid profile. It remains unknown whether adverse cardiovascular outcomes increase when HDL cholesterol is decreased in the context of an ultra low-fat diet. There was no statistically significant inverse change in triglycerides at one year, related to the wide variability usually found in measurement of this parameter. Similar findings in standard lipid measurements have been reported from the Ornish Multicenter Lifestyle Demonstration Project. In 333 patients at three months, HDL cholesterol decreased 11% and LDL decreased by 14% from baseline values.[10]

After 3 months, patients increased their cardiovascular fitness level by 1.7 METS (metabolic equivalent). Twelve-month data shows improvement of this workload at a significant level. There

is evidence-based data that an increase of 1-MET in functional capacity may convey a 12% increase in survival.[11]

Participants have done remarkably well in integrating this ultra-low fat diet into their daily routine. Significant reductions in weight and BMI have also been seen as a result of the diet, regular exercise and frequent clinical monitoring. There was a 7% reduction in weight and BMI during the first 12 weeks. During the last two phases of the program (less frequent clinical monitoring), there appears to be a small decrease in program adherence. This decrease in adherence may have resulted in the slight increase in body composition (weight & BMI) at one-year. However, the percent of weight loss and reduction in BMI over the course of the study was statistically significant.

Functional health improvement has also been validated in this population through the use of the Health Status Survey (SF-36, v.1), which is a widely used tool for measuring health status and outcomes. Improvements were seen in both the physical and mental components of this tool. Those participants with coronary heart disease also were able to improve or maintain their physical limitations according to the NYHA class. At the conclusion of the study, over 98% of those with CHD had either no physical limitations or slight limitations. The functional improvements in overall health, both physical and mental, that participants were able to make during the course of the study may be attributable to regular clinical monitoring as well as intensive lifestyle changes.

Task #2: Initiate "A Blood Repository for Analysis of Molecular Changes Associated with Cardiovascular Disease Development" protocol.

Status: A draft protocol entitled "A Blood Repository for Analysis of Molecular Changes Associated With Cardiovascular Disease Development" has been developed in collaboration with WRI. This protocol was submitted to WRAMC DCI on 20 June 05 and was approved with revisions by the WRAMC Clinical Investigations Committee (CIC) on 19 July 05. The protocol was initially reviewed by the WRAMC Human Use Committee (HUC) on 23 August 05. The HUC requested substantive revisions and was tabled. Currently, WRAMC and WRI working collaboratively to clarify issues raised by WRAMC HUC (ie. WRI Tissue Banking policy and Relational Database).

Task #3: Initiate CADRe Five-Year Follow-up Protocol.

Status: WRAMC HUC approved this study on 23 May 2006. Protocol is currently at US Army Medical Department Center and School, Clinical Investigations Regulatory Office (CIRO) awaiting final approval.

Study Design and Objectives:
This follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control results after their original CADRe study participation. This study will continue as a longitudinal observational study where patients will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. This study will involve prospective collection of data, however, there will be no tests ordered that are not considered WRAMC Cardiology standard of care for the study population identified. Therefore, there are no risks involved with this study outside those of the standard of care treatment. Specific aims are to determine:

1. Persistence of lifestyle change behaviors in diet, exercise, and stress management
2. Coronary risk-factor control

3. Quality of Life

Hypothesis
Subjects who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control.

Up to 163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) will be recontacted and invited to participate in this five (5) year follow-up study (post-study completion or expected completion). Because of the timing of this protocol submission, the earlier cohorts will not have as many yearly follow-up periods as the later cohorts (See Table 6). The final cohort of participants completed the CADRe study in April 2005.

Table 6. Projected Longitudinal Follow-Up of CADRe Study

Cohort #	Completers (≥ 12 weeks of intervention)	Dropouts (< 12 week exposure)	No longer available	Available to enroll	Available Follow-Up				
					1-Yr	2-Yr	3-Yr	4-Yr	5-Yr
1	7	3		7					X
2	16	2	1	15					X
3	15	5	1	14					X
4	18	2	1	17				X	X
5	12	1		12				X	X
6	19			19				X	X
7	15	5		15			X	X	X
8	12	4		12			X	X	X
9	9	2		9			X	X	X
10	10			10		X	X	X	X
11	11	1		11		X	X	X	X
12	10	4		10		X	X	X	X
13	12	5		12	X	X	X	X	X
Totals	166	34	3	163	12	43	79	127	163

Primary Outcome Measure - Heart Health Index (HHI)
A composite index of 7 heart healthy characteristics (BMI 18.5 – 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise ≥ 150 min/week, and daily practice of CADRe program stress management techniques) was selected as the primary outcome measure since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The HHI, presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable.

Secondary Outcomes
Several additional outcomes will be assessed including:

- Changes in modifiable CVD risk factors: blood pressure, body composition and fitness, lipid levels and glucose
- Other biochemical markers: C-reactive protein
- Quality of Life: SF-36

Task #4: Initiate "*B*etter Adherence *t*o *T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".

Status: WRAMC HUC approved protocol on 25 April 2006 and forwarded study to CIRO for final approval. CIRO determined COL(ret) Vernalis, Henry M. Jackson Foundation employee, under AR 40-38 was ineligible to act as study PI and requested PI change to a **WRAMC-assigned** investigator. Approval received for COL Allen Taylor, Chief, WRAMC Cardiology Services, to assume the PI role and COL(ret) Marina Vernalis assume the AI role. Final study approval from CIRO was granted on 28 April 2006. Study team is actively working on CRFs and the Operations Manual in preparation for study initiation. Staff acquisition (sonographer/research nurse) and training as also in progress. The contractual agreement process for data management and lab testing services has begun. Plan to enroll subjects beginning October 2006.

The **B**etter **A**dherence **t**o **T**herapeutic **L**ifestyle Change **E**fforts (**BATTLE**) Trial is a two-arm, randomized, clinical trial that will determine if CIMT ultrasound images motivate adherence to a therapeutic lifestyle change (TLC) intervention program involving a Mediterranean-type diet, exercise, and group support. The study population will have CVD risk factors and significant subclinical atherosclerosis, determined by carotid ultrasound images of carotid intima-medial thickness (CIMT). All subjects will participate in the same TLC program. However, one group of subjects will receive their CIMT results at the beginning of the TLC program and the other group will receive these results after completion of the study. The primary outcome measure is the change in a composite score for adherence with the TLC intervention. Other outcomes to be studied include: adherence to individual TLC program components, change in CVD risk factors, inflammatory and diabetes-related metabolic markers, and anxiety. This study will provide new scientific evidence on the value of CIMT testing as a motivational tool for individuals seeking lifestyle change training in the setting of a cardiac prevention clinic.

A protocol addendum was submitted to WRAMC DCI on 24 July 2006. The following proposed changes to this study are as follows:

1) Additional advertisements (simplified version of approved advertisement specifically for newspaper and electronic media)
2) Expanded version of Mediterranean Diet Short Dietary Intake Questionnaire
3) Inclusion of self-efficacy and motivation measures
4) Location change of laboratory performing Omega-3 Index

Task #5: Ongoing enrollment to Dr. Dean Ornish Program for Reversing Heart Disease protocol.

Status: Study is currently ongoing.

Background: The Dr. Dean Ornish Program for Reversing Heart Disease
The Ornish Program, an intensive lifestyle modification program, was established in January 2000 at Windber Medical Center (WMC). The objective of the study at WMC is to determine the one-year efficacy of the intensive lifestyle modification program with a focus on diet, exercise, stress management, and group support for improving the clinical status of patients with moderate to severe coronary artery disease or risk factors that would promote coronary heart disease (CHD). Outcome measures include: (1) biochemical/anthropometric measures: blood lipids, glucose, glycosylated hemoglobin, blood pressure, weight, body composition, body mass index (BMI), and exercise capacity achieved on exercise stress testing; (2) psychometric test scores: depression, hostility, preferred support, perceived stress, and quality of life; and (3)

adherence outcome measures: retention and attrition rates, attendance, and overall and individual adherence scores for stress management (7 days a week for a total of 420 minutes), moderate aerobic exercise (180 minutes per week), and diet (low-fat vegan diet).

Subject Enrollment and Demographics

Subject enrollment to date is 368 participants including 20 cohorts and 4 retreats. There are 28 subjects who continue to actively participate in the year-long program, 306 have graduated, and 62 have discontinued participation (17% dropout rate). Demographic characteristics of participants are: average age of 63.9 years, 52% are female, 34% are veterans or the spouse of a veteran, and 40% have diagnosed coronary heart disease.

Subject Adherence

Experience throughout the study over the past six years has shown that subjects are able to make and maintain comprehensive changes in nutrition and lifestyle over a minimum period of one year (Table 7). During their entire year of participation, subjects in the WMC Ornish Program are required to complete a Personal Awareness Log (PAL form) weekly, which includes daily documentation of nutritional intake, stress management, exercise, and group support and is reviewed weekly by the clinical staff. Weekly staff meetings are held to discuss each subject's medical status and adherence, and clinical staff provide feedback to assist and encourage subjects to increase adherence to the defined guidelines as required.

Table 7. Adherence to dietary, exercise, stress management, and group support guidelines over 12-week and one-year periods for participants in the Ornish Program for Reversing Heart Disease at Windber Medical Center*

Guideline	Compliance goal (%)	12 Weeks		1 Year	
		Average level % compliance (n=224)	% of goal	Average level % compliance (n=224)	% of goal
Diet (% compliance)†	100	94.88	94.88	95.64	95.64
Stress management (% compliance)	100	100.06	100.06	100.72	100.72
Exercise (% compliance)	100	122.37	122.37	116.95	116.95
Group support (% attendance)	80	96.65	120.81	88.29	110.36
Total adherence score‡	--	--	109.53	--	105.91

* Includes the most recent relevant data from Cohorts #1 through #20 as of July 31, 2006; excludes subjects who discontinued participation in the program.

† Diet - compliance measured as a percentage of the recommended diet goals actually achieved; stress management - compliance is a percentage of the recommended level (one hour/day, seven days/week) actually attained; exercise - compliance is the percentage of the recommended level (180 min/week) actually achieved; group support - compliance is the percentage of sessions attended.

‡ Calculated as the average percentage of lifestyle changes achieved by participants at the 12-week and one-year examinations.

Outcome Data

Participants in the Dr. Dean Ornish Program at Windber Medical Center have achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period (Table 8A). Measures of obesity including weight and BMI declined 7%, levels of total cholesterol were reduced by nearly 14%, blood pressure dropped 10%, measures of physical fitness increased more than 25%, and levels depression decreased approximately 50%. These data demonstrate that lifestyle change programs may be

important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Results from the end of the year examination are shown in Table 8B. Over the course of one year, weight, BMI, and LDL-cholesterol decreased ~8%, diastolic blood pressure decreased 6%, measures of physical fitness increased 30%, and levels of depression decreased more than 50%.

Table 8A. Change in Outcome Variables after 12 weeks for 215 Participants in the Lifestyle Change Program for Heart Disease Reversal

Category / Metrics	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
Weight (lbs.)	215	203.83 (44.5)	190.39 (39.4)	-13.4	<0.00001
Body Mass Index	213	32.30 (6.8)	30.21 (6.0)	-2.1	<0.00001
Total Cholesterol (mg/dl)	215	194.09 (42.2)	167.12 (37.3)	-27.0	<0.00001
High Density Lipids (mg/dl)	215	46.93 (12.7)	40.76 (9.5)	-6.2	<0.00001
Low Density Lipids (mg/dl)	204	110.12 (35.0)	92.46 (29.9)	-17.7	<0.00001
Triglycerides (mg/dl)	215	186.23 (99.8)	169.92 (81.8)	-16.3	<0.01
Systolic Blood Pressure	215	136.72 (18.2)	123.68 (15.3)	-13.0	<0.00001
Diastolic Blood Pressure	215	80.86 (10.6)	72.61 (8.6)	-8.2	<0.00001
Exercise Capacity (min.) [BRUCE]	213	7.02 (2.7)	8.92 (2.7)	1.90	<0.00001
Oxygen Capacity [METS]	212	8.40 (2.7)	10.24 (2.8)	1.84	<0.00001
Depression Scale [CES-D]	215	12.16 (10.2)	6.23 (6.1)	-5.9	<0.00001
Hostility Scale [Cook-Medley]	215	8.26 (4.7)	6.17 (4.3)	-2.1	<0.00001
Daily Total Fat (grams)	209	62.59 (38.2)	18.23 (6.0)	-44.4	<0.00001
Daily Saturated Fat (grams)	209	19.33 (13.4)	3.25 (1.5)	-16.1	<0.00001
% Daily Caloric Fat Intake	209	27.89 (9.7)	10.50 (2.9)	-17.4	<0.00001

Table 8B. Change in Outcome Variables after one year for 194 Participants in the Lifestyle Change Program for Heart Disease Reversal

Category / Metrics	N	Mean Baseline (SD)	Mean Year 1 (SD)	Mean Change	P
Weight (lbs.)	193	201.03 (44.1)	183.73 (39.4)	-17.3	<0.00001
Body Mass Index	187	31.95 (6.8)	29.25 (6.0)	-2.7	<0.00001
Total Cholesterol (mg/dl)	194	193.63 (41.6)	180.99 (38.6)	-12.6	<0.00001
High Density Lipids (mg/dl)	194	47.66 (12.8)	46.39 (13.1)	-1.3	0.0990
Low Density Lipids (mg/dl)	184	109.30 (33.7)	100.46 (31.2)	-8.8	<0.0001
Triglycerides (mg/dl)	194	185.92 (98.2)	174.06 (84.8)	-11.9	<0.05
Systolic Blood Pressure	194	135.66 (18.3)	127.02 (19.0)	-8.6	<0.00001
Diastolic Blood Pressure	194	80.23 (10.7)	74.98 (9.6)	-5.2	<0.00001
Exercise Capacity (min.) [BRUCE]	188	7.20 (2.7)	9.27 (3.0)	2.08	<0.00001
Oxygen Capacity [METS]	188	8.51 (2.7)	10.57 (3.0)	2.06	<0.00001
Depression Scale [CES-D]	191	12.14 (10.0)	5.95 (5.7)	-6.2	<0.00001
Hostility Scale [Cook-Medley]	191	8.31 (4.7)	5.82 (4.2)	-2.5	<0.00001
Daily Total Fat (grams)	171	62.43 (37.2)	22.35 (9.7)	-40.1	<0.00001
Daily Saturated Fat (grams)	171	19.56 (13.8)	4.21 (2.5)	-15.4	<0.00001
% Daily Caloric Fat Intake	185	27.51 (9.8)	11.67 (4.0)	-15.8	<0.00001

In subjects matched for age, gender, and disease status who did not participate in the Ornish lifestyle change program, most risk factors did not show significant changes after 12 weeks in the Program (Table 9A). Only blood pressure and exercise capacity differed between the baseline and 12-week examinations. At the end of one year, all variables were not significantly different from baseline (Table 9B).

Table 9A. Change in Outcome Variables after 12 Weeks for 56 Subjects Matched for Age, Gender and Disease Status Who Did Not Participate in a Lifestyle Change Program

Category / Metrics	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
Weight (lbs.)	56	181.63 (30.4)	180.73 (30.2)	-0.9	0.2318
Body Mass Index	12	29.60 (3.4)	29.43 (3.2)	-0.2	0.4725
Total Cholesterol (mg/dl)	55	194.95 (43.7)	191.95 (40.9)	-3.0	0.5082
High Density Lipids (mg/dl)	55	55.42 (13.0)	55.18 (12.8)	-0.2	0.8091
Low Density Lipids (mg/dl)	53	112.08 (35.6)	108.42 (34.5)	-3.7	0.3153
Triglycerides (mg/dl)	55	140.05 (103.7)	142.55 (84.2)	2.5	0.7894
Systolic Blood Pressure	56	136.36 (16.1)	130.32 (13.7)	-6.0	<0.01
Diastolic Blood Pressure	56	80.75 (9.6)	78.32 (7.8)	-2.4	<0.05
Exercise Capacity (min.) [BRUCE]	52	10.07 (2.8)	10.39 (2.7)	0.32	<0.05
Oxygen Capacity [METS]	52	11.22 (2.5)	11.69 (2.4)	0.48	<0.01
Depression Scale [CES-D]	55	5.13 (6.5)	5.44 (7.5)	0.3	0.6142
Hostility Scale [Cook-Medley]	55	7.55 (4.1)	7.44 (4.2)	-0.1	0.8103
Daily Total Fat (grams)	20	67.90 (30.7)	62.93 (24.7)	-5.0	0.2840
Daily Saturated Fat (grams)	20	22.39 (10.9)	20.72 (7.6)	-1.7	0.3197
% Daily Caloric Fat Intake	20	32.85 (10.9)	32.25 (7.3)	-0.6	0.6609

Table 9B. Change in Outcome Variables after One Year for 56 Subjects Matched for Age, Gender and Disease Status Who Did Not Participate in a Lifestyle Change Program

Category / Metrics	N	Mean Baseline (SD)	Mean Year 1 (SD)	Mean Change	P*
Weight (lbs.)	38	184.75 (31.8)	186.05 (34.4)	1.3	0.2871
Body Mass Index	0	---	---	---	---
Total Cholesterol (mg/dl)	37	192.70 (37.3)	194.14 (42.5)	1.4	0.7823
High Density Lipids (mg/dl)	37	54.46 (11.1)	52.22 (14.0)	-2.2	0.0601
Low Density Lipids (mg/dl)	36	112.08 (32.0)	112.06 (36.1)	0.0	0.9952
Triglycerides (mg/dl)	37	138.97 (104.0)	154.11 (104.4)	15.1	0.1023
Systolic Blood Pressure	38	133.58 (15.9)	130.11 (14.0)	-3.5	0.1591
Diastolic Blood Pressure	38	80.05 (9.9)	78.21 (9.2)	-1.8	0.2499
Exercise Capacity (min.) [BRUCE]	31	10.35 (2.8)	10.57 (3.0)	0.22	0.2779
Oxygen Capacity [METS]	31	11.50 (2.5)	11.97 (2.8)	0.47	0.1367
Depression Scale [CES-D]	37	5.11 (6.2)	6.95 (7.1)	1.8	0.0757
Hostility Scale [Cook-Medley]	37	8.00 (4.2)	7.78 (4.1)	-0.2	0.6949

Many patients showed a biphasic response to the Ornish program for certain physical risk factors, achieving rapid improvement in the risk factor variables during the initial 12-week period, but then partial reversal in risk factor levels at the end of the year (Figure 4). Systolic blood pressure and most biochemical variables improved during the initial 12-week period but then reversed some of their early gains by the end of one year. Conversely, the psychometric measures retained their level of improvement or continued to improve from 12-weeks to one year.

Significant changes in the distributions of participants with clinically-significant conditions were observed for depression, stress, and the mental health composite score (MCS) from baseline to 12 weeks and from baseline to one year ($p<0.001$) (Table 10). In all cases, the number of participants with a clinically-significant condition decreased significantly from baseline to 12 weeks and baseline to one year. Gender was not a significant factor for clinical changes in depression, stress, and mental health scores from baseline to 12 weeks and was not a significant factor for clinical changes in stress and mental health from baseline to one year. However, men and women responded differently with respect to clinical depression by one year. Although the reduction in the number of clinically-depressed women was significantly greater than that observed in men, there were nearly twice as many women with clinical depression at baseline. Due to the small number of participants considered hostile at baseline, no significant clinical changes were observed for hostility.

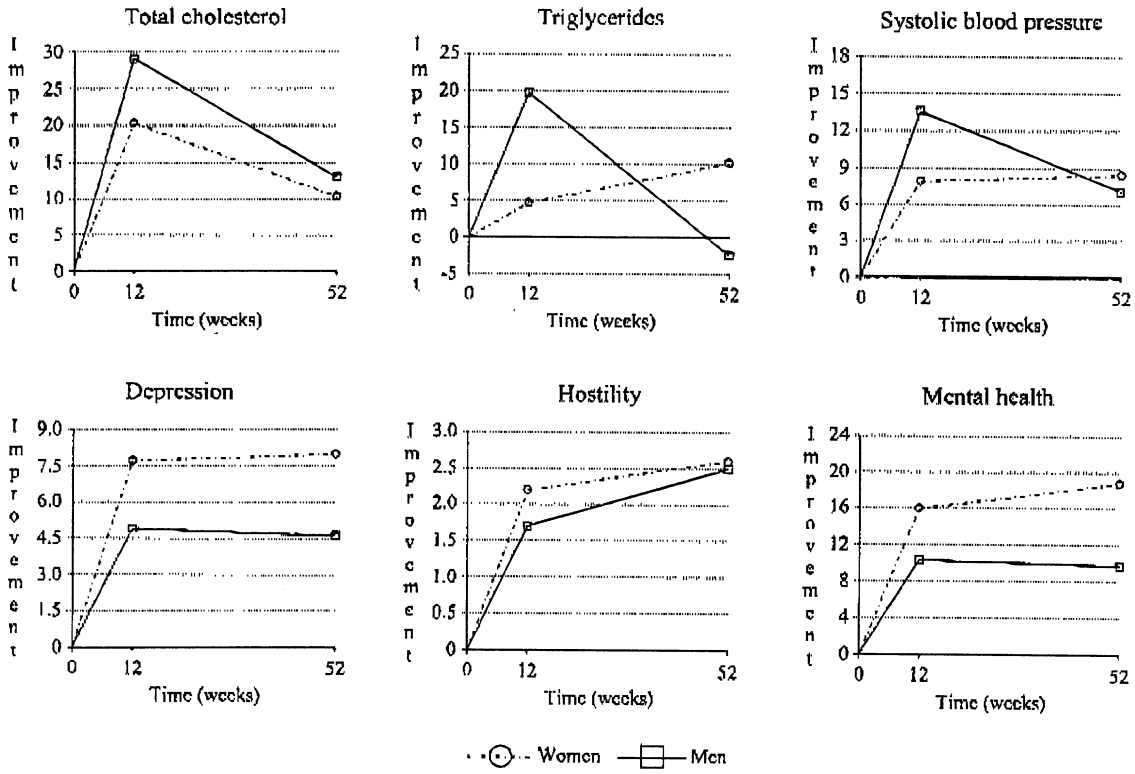


Figure 4. Improvement in selected CAD risk factor levels after 12 Weeks and One Year among participants in the a CAD Reversal Program stratified by gender.

Table 10. Clinical Changes in Psychometric Variables for Participants in a Lifestyle Change Program for Heart Disease Reversal

Psychometric Variable	Baseline to 12 Weeks	*P*-value[a]	Baseline to One Year	*P*-value[b]
Depression scale (CESD)				
Depressed → Not depressed	43		48	
Remained depressed	10		5	
Not depressed → Depressed	6		7	
Remained not depressed	113	<0.001	112	<0.001
Perceived stress scale				
Stressed → Not stressed	53		57	
Remained stressed	14		10	
Not stressed → Stressed	3		0	
Remained not stressed	102	<0.001	105	<0.001
Hostility Scale (Cook-Medley)				
Hostile → Not hostile	6		6	
Remained hostile	1		1	
Not hostile → Hostile	2		3	
Remained not hostile	163	0.199	162	0.358
Mental health composite score				
Below average → Average or above	46		48	
Remained below average	9		7	
Average or above → Below average	3		4	
Remained average or above	114	<0.001	113	<0.001

[a] Results from Chi-square testing of a 2 x 2 contingency table containing counts of participants above versus below established thresholds for risk stratification at baseline and 12-weeks.

[b] Results from Chi-square testing of a 2 x 2 contingency table containing counts of participants above versus below established thresholds for risk stratification at baseline and one-year.

Adverse Events

All adverse events are submitted and adjudicated by the Windber Medical Center Institutional Review Board and USUHS Office of Research after review by both the Principal Investigator and Medical Monitor. There have been 81 adverse events over the course of the study, of which 56 were serious events and 25 were considered non-serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, the 56 events were considered serious due to inpatient hospitalizations. There were 51 non-cardiac and 30 cardiac adverse events. No deaths occurred and none of these adverse events were deemed to be study related.

Task #6: Ongoing enrollment to Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Subjects in the Dr. Dean Ornish Program protocol.

Status: Enrollment to both global profiling studies is ongoing.

1. Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal. This study is characterizing: 1) longitudinal changes in gene and protein expression in blood during an intensive lifestyle intervention; 2) associations of gene/protein expression profiles and single nucleotide polymorphisms (SNPs) with changes in quantitative CHD risk factors/CHD status during the intervention; and 3) gene/protein expression profiles and SNPs predictive of significant differences among individuals in patterns of change in CHD risk factors and CHD phenotypes during the lifestyle change program. This research has the potential to provide a global view of molecular changes associated with lifestyle modifications designed to reverse CHD and improve our understanding of molecular events crucial to CHD development.

Collaborators and external relationships for this project include Marina Vernalis, Debra Marshall, Elaine Walizer at Walter Reed Army Medical Center, and LipoScience, Inc., the Department of Endocrinology, University of Maryland, Baltimore, Johns Hopkins University, and Josef Machac, MD, at Mt. Sinai Medical Center.

Subject Enrollment and Demographics
Subject enrollment to date is 271. There are 112 participants taking part in the lifestyle change program and 91 subjects enrolled thus far serving as the control group. Demographic characteristics of the control group are: average age of 61.7 years, 49% are female, 25% are veterans or the spouse of a veteran, and 35% have diagnosed coronary heart disease.

Sample Collection
From July 1, 2005 to June 30, 2006, there were 19 examinations and blood draws for Ornish Program and control group participants. During the year, approximately 5,518 aliquots of biological material were collected as summarized in the following table:

Cohorts and Research Group Examinations	19	
Individual Patients	235	
PAXGene Tubes	260	
Plasma Samples		3,564
Total Aliquots		5,518

NMR Lipid Panel
Participants experienced clinically-important improvements in a number of components of the NMR lipid panel, which gives detailed information on particle size and number for VLDL, HDL, and LDL (Table 11). These data are important, because several studies have linked selected variables, such as LDL particle number more strongly to CAD risk than LDL-cholesterol. Recommended LDL-PN goals are <1000 nmol/L (<20th percentile) for high risk patients and <1300 nmol/L (<50th percentile) for moderately high-risk patients. As previously observed for selected biochemical variables, some of the NMR lipid panel variables also regress toward baseline values by the end of one year (Table 12).

Table 11. Change in NMR Lipids After 12 Weeks in Participants in the Lifestyle Change Program for CAD Reversal

Category	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
VLDL Particle Concentrations (nmol/L)					
VLDL Particles (total) [VLDLP]	90	89.90 (40.5)	98.56 (44.2)	8.7	<0.01
Large VLDL/Chylomicrons [VL]	90	8.87 (7.2)	5.91 (6.2)	-3.0	<0.001
Medium VLDL [VM]	90	39.88 (24.6)	51.92 (32.7)	12.0	<0.0001
Small VLDL [VS]	90	41.16 (20.7)	40.73 (18.1)	-0.4	0.8448
LDL Particle Concentrations (nmol/L)					
LDL Particles (total) [LDLP]	90	1463.86 (469.6)	1258.19 (451.5)	-205.7	<0.00001
IDL	90	78.46 (54.1)	48.39 (44.0)	-30.1	<0.00001
Large LDL [LL]	90	233.76 (202.9)	203.42 (151.5)	-30.3	0.0516
Small LDL (total) [LS]	90	1151.63 (459.9)	1006.41 (429.3)	-145.2	<0.001
Medium Small LDL [LMS]	90	240.58 (93.5)	205.99 (89.5)	-34.6	<0.0001
Very Small LDL [LVS]	90	910.97 (370.9)	800.44 (342.9)	-110.5	<0.001
HDL Particle Concentrations (µmol/L)					
HDL Particles (total) [HDLP]	90	32.78 (5.8)	28.98 (4.8)	-3.8	<0.00001
Large HDL [HL]	90	4.89 (3.3)	4.26 (2.3)	-0.6	<0.01
Medium HDL [HM]	90	5.52 (4.5)	4.12 (4.2)	-1.4	<0.001
Small HDL [HS]	90	22.38 (6.3)	20.60 (4.9)	-1.8	<0.01
Mean Particle Sizes (nm diameter)					
VLDL Size [VZ]	90	57.39 (10.3)	49.48 (8.6)	-7.9	<0.00001
LDL Size [LZ]	90	20.17 (0.7)	20.20 (0.6)	0.0	0.6561
HDL Size [HZ]	90	8.65 (0.3)	8.68 (0.3)	0.0	0.1669
Calculated Lipids (mg/dL)					
Triglycerides [NTG]	90	195.63 (93.7)	172.73 (82.6)	-22.9	<0.01
VLDL-TG [NVTG]	90	156.74 (92.2)	141.14 (81.4)	-15.6	0.0729
HDL-C [NHDLC]	90	44.23 (11.5)	38.88 (8.3)	-5.4	<0.00001

Table 12. Change in Selected NMR Lipids After One Year in Participants in the Lifestyle Change Program for CAD Reversal

Category	N	Mean Baseline (SD)	Mean Year 1 (SD)	Mean Change	P
VLDL Particle Concentrations (nmol/L)					
VLDL Particles (total) [VLDLP]	53	94.70 (44.7)	97.77 (42.5)	3.1	0.5832
Large VLDL/Chylomicrons [VL]	53	9.09 (7.0)	5.79 (6.7)	-3.3	<0.001
Medium VLDL [VM]	53	42.03 (27.3)	48.00 (29.6)	6.0	0.1117
Small VLDL [VS]	53	43.58 (22.5)	43.99 (22.3)	0.4	0.9028
LDL Particle Concentrations (nmol/L)					
LDL Particles (total) [LDLP]	53	1464.30 (457.6)	1302.60 (451.7)	-161.7	<0.001
IDL	53	82.51 (55.4)	62.53 (50.8)	-20.0	<0.05
Large LDL [LL]	53	242.34 (229.4)	251.57 (188.9)	9.2	0.6593
Small LDL (total) [LS]	53	1139.49 (453.7)	988.49 (484.6)	-151.0	<0.01
Medium Small LDL [LMS]	53	236.45 (90.8)	206.87 (108.2)	-29.6	<0.01
Very Small LDL [LVS]	53	902.98 (369.2)	781.60 (380.8)	-121.4	<0.01
HDL Particle Concentrations (µmol/L)					
HDL Particles (total) [HDLP]	53	33.64 (5.7)	33.16 (5.1)	-0.5	0.5341
Large HDL [HL]	53	5.07 (3.8)	5.27 (3.2)	0.2	0.5688
Medium HDL [HM]	53	5.64 (4.6)	4.94 (4.9)	-0.7	0.2711
Small HDL [HS]	53	22.93 (6.4)	22.95 (5.8)	0.0	0.9780
Mean Particle Sizes (nm diameter)					
VLDL Size [VZ]	53	57.13 (9.5)	49.51 (9.1)	-7.6	<0.00001
LDL Size [LZ]	53	20.18 (0.8)	20.40 (0.8)	0.2	<0.01
HDL Size [HZ]	53	8.67 (0.3)	8.70 (0.4)	0.0	0.3931
Calculated Lipids (mg/dL)					
Triglycerides [NTG]	53	202.21 (97.3)	172.85 (88.0)	-29.4	<0.01
VLDL-TG [NVTG]	53	162.49 (96.0)	137.04 (85.5)	-25.5	<0.05

Inflammatory Markers

Assays are being conducted for a panel of five inflammatory markers, potentially important in CAD development and/or reversal: insulin, leptin, C-reactive protein, interleukin-6, and interleukin-8. Preliminary results are shown in Tables 13A-13B. Leptin levels decrease significantly in Ornish participants after 12 weeks; leptin, interleukin-6, and interleukin-8 all decrease significantly after one year. Only leptin levels decreased significantly in control subjects, but this change will be evaluated in the coming year as data from more samples are collected.

Table 13A. Change in Inflammatory Markers After 12 Weeks in Ornish Program Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	41	19.89 (11.5)	16.80 (10.7)	-3.089	0.1571
HS-CRP [ug/ml	41	6.26 (8.0)	4.29 (4.6)	-1.971	0.0817
Leptin [ng/ml]	41	29.55 (20.2)	17.82 (12.3)	-11.734	<0.0001
IL-6 [pg/ml]	41	2.99 (1.7)	3.04 (2.1)	0.048	0.8899
IL-8 [pg/ml]	39	14.37 (3.4)	15.24 (4.6)	0.871	0.0867

Table 13A. Change in Inflammatory Markers After One Year in Ornish Program Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	10	15.16 (8.4)	13.75 (9.3)	-1.403	0.2808
HS-CRP [ug/ml	10	3.65 (3.7)	2.98 (3.6)	-0.666	0.4542
Leptin [ng/ml]	10	23.07 (15.4)	11.99 (7.8)	-11.083	<0.01
IL-6 [pg/ml]	9	3.53 (1.9)	1.53 (0.8)	-2.004	<0.05
IL-8 [pg/ml]	10	13.69 (3.0)	11.28 (1.9)	-2.409	<0.05

Table 13C. Change in Inflammatory Markers After 12 Weeks in Control Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	38	15.88 (9.3)	17.65 (7.4)	1.768	0.2888
HS-CRP [ug/ml	39	3.13 (3.8)	3.35 (3.6)	0.211	0.7460
Leptin [ng/ml]	39	19.91 (12.7)	15.19 (9.6)	-4.719	<0.0001
IL-6 [pg/ml]	39	1.67 (1.0)	1.92 (1.5)	0.245	0.3565
IL-8 [pg/ml]	32	12.73 (4.1)	13.63 (3.3)	0.901	0.2699

Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) are derived from bone marrow and function in ongoing endothelial repair. Impaired mobilization or depletion of these cells has been shown to contribute to endothelial dysfunction and cardiovascular disease progression. The number of endothelial progenitor cells in peripheral blood is being assessed to determine how numbers of these cells change in response to the program. Preliminary results are provided in Tables 14A and 14B.

Thus far, significant changes in levels of endothelial progenitor cells have been detected for both Ornish participants and control subjects at the 12-week examination. Although control subjects are carefully matched to Ornish participants for age, gender, and disease status, control subjects have much higher levels of EPCs at baseline. Despite this difference, a pattern that is beginning to emerge is that EPC numbers decrease significantly in control subjects by the end of the year, but do not change significantly in Ornish participants.

Table 14A. Change in Endothelial Progenitor Cells after 12 Weeks in Ornish Program and Control Participants

Category	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
Ornish Participants	60	0.118 (0.070)	0.092 (0.053)	-0.025	<0.001
Controls	43	0.183 (0.134)	0.136 (0.086)	-0.048	<0.05

Table 14B. Change in Endothelial Progenitor Cells after 1-Year in Ornish Program and Control Participants

Category	N	Mean Baseline (SD)	Mean One Year (SD)	Mean Change	P
Ornish Participants	31	0.108 (0.062)	0.091 (0.049)	-0.017	0.206
Controls	20	0.217 (0.162)	0.128 (0.071)	-0.089	<0.05

Gene Expression

At the beginning of the year, RNA was isolated from PAXgene tubes for 11 Ornish participants, who had one PAXgene tube for each of the following timepoints: baseline, 3 months, and 1 year. After purifying RNA samples, the CodeLink Gene Expression System (then in place at the WRI) was used to process the samples. Due to ongoing problems associated with reproducibility on the CodeLink platform – encountered in both the Molecular and Clinical Based Comprehensive Cardiac Care Program and the Clinical Breast Care Program – we began testing the Affymetrix platform, purchased by the CVD program for SNP genotyping, and switched to this system due to QA/QC data that are superior to that of CodeLink.

Beginning in November 2005, considerable work focused on developing new standard operating protocols for gene expression on the Affymetrix platform. As mentioned above, all previous gene expression analysis had been done through protocols developed by the core facility at WRI using Codelink assays from GE Healthcare. Three Ornish patient RNA samples were initially selected to analyze via the Affymetrix gene expression protocol. Samples were chosen from one patient (ID 281) collected at three time points: baseline, 3 months, and 1 year, and processed to completion according to the Affymetrix gene expression protocol. Resultant data indicated that technical aspects of the protocol worked well, but based on a number of Affymetrix internal controls that monitor the success of various steps in the protocol, it was determined that the number of probe sets called as present by the analysis software was below 30%. Average to high call rates should be ~40-60% and a reduced percentage of present call rates may be due to the presence of globin in the total RNA that is isolated from PAXgene tubes. Therefore, a globin reduction protocol was evaluated in combination with the normal gene expression protocol.

Globin reduction must be performed on RNA that is isolated from blood, because the presence of globin may significantly interfere with the expression of some genes. Since globin accounts for ~70% of the RNA present in total RNA isolated from blood, globin reduction would increase the recognition of genes that are expressed at lower levels. The Affymetrix globin reduction protocol involves the use of peptide nucleic acids (PNAs), which must be handled carefully to fully reconstitute. Due to difficulties with this protocol of trying to get the PNAs solubilized to the correct concentration, a similar globin reduction protocol from another commercial vendor, Ambion (GLOBINclear™) was also tested. After sufficient evaluation as outlined below, data from the Affymetrix assays indicated that the globin was reduced successfully using the GLOBINclear™ and the percent present call rates on the gene expression reports increased to above 50%.

During the months of January and February, 17 samples of PAXgene RNA were analyzed using the Affymetrix gene expression protocol, 12 were performed with the globin reduction step, and 5 were performed without globin reduction. All 17 trials were successful as determined by the internal controls.

In early February statistical analyses were conducted to assess the performance of gene expression from samples that did or did not receive globin reduction. Seven total comparisons were made between paired samples (3 experimental human samples and one duplicate). Probe sets determined to be "present" were found by Wilcoxon's rank test to be statistically significant. Therefore, an increase of "present" calls increases the number of genes that can be detected per experiment, providing more detailed and potentially important molecular information per experiment. In the experimental human samples, globin reduction increased the percentage of probe sets called "present" by ~15% on average. The duplicate performed extremely well differing in "present" calls by <0.3%.

As mentioned above, gene expression data using two techniques to reduce the number of globin transcripts, globin reduction (6 chips; 3 samples) (Affymetrix) and GLOBINclear™ (10 chips; 5 samples) (Ambion) were obtained. Reports for the arrays were generated to determine the proportion of present/marginal/absent calls as well as fold-change in gene expression levels. Distributions were created for genes that exhibited consistent expression (present-present, absent-absent, marginal-marginal) across all samples in order to search for intensity-related issues, using caGEDA. Genes for which the ability to detect their expression changed as a result of applying globin reduction procedures (for example, genes absent prior to globin reduction but present after globin reduction, marginal → present, marginal → absent, present → absent) from all five experiments were identified and grouped. Genes containing the root 'globin' in their description were also identified to observe changes in their expression. Genes that consistently changed across all five samples were considered of greatest interest for further study. Of these, gene ontologies consisting of cellular location, molecular function, and biological process were summarized for further classification. Public "normal" data was used in order to determine "normal" expression levels for each gene and compared to our sample data. Lastly, Spotfire™ was used for hierarchical clustering of all data to determine expression change. Graphs, charts, and lists were created for presentation purposes and a summary of all procedures is currently being prepared for publication.

The GLOBINclear protocol was implemented to reduce the globin transcripts in total RNA isolated from PAXgene tubes, and in combination with Ambion's MessageAmp II aRNA amplification protocol, was used to successfully amplify RNA in preparation for gene expression array analysis. Globin-depleted RNA samples from the ten Ornish participants with three time points each (30 samples) mentioned above were assayed on GeneChip® Human Genome U133A 2.0 arrays (Affymetrix), which assay ~14,000 genes. Call rates using the globin reduction protocol were all >50%.

Analysis of these initial samples is in progress. Thus far, we have observed approximately 1,200 genes that are differentially expressed in peripheral blood between the three examinations (baseline, 12 weeks, one-year). Preliminary results indicate that many of the genes changing in expression during participation in the program are:

1. protein binding
2. metal ion binding
3. DNA/nucleotide binding
4. receptors
5. hydrolases
6. transferases

These results suggest that fundamental molecular changes occur due to participation in the program. Continuing studies will examine the functional biology of these genes and their relationship with risk factor response.

Structural and Functional Measures of Cardiovascular Health

The Windber Medical Center acquired a new 16-slice combination PET/CT imaging system in 2004 to obtain detailed quantitative images to quantify a number of important measures of cardiovascular disease. The HeartFusion Cardiology Applications provide comprehensive assessments of coronary disease by combining CT angiography with PET data to simultaneously provide anatomical and functional information.

Specific endpoints we are measuring include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. Data from the combination PET and coronary angiography scans are being acquired through a collaboration with Dr. Josef Machac at Mt. Sinai Medical Center in New York, who is joining our group as a collaborator to assist us in the quantification and interpretation of the huge volumes of imaging data we have acquired. To date, we have conducted on Ornish participants (includes PET rubidium, Calcium CT, and CTA scans) 49 baseline, 31 12-week, and 18 one-year scans and on control participants, 50 baseline, 17 12-week, and 12 one-year scans. As these images are read by Dr. Machac's group and the research data are collected in the coming months, a comprehensive picture of the effects of the program on cardiac health will emerge.

Proteomics

During the year, extensive efforts and progress were made in cardiovascular protein expression profiling. The primary component in this effort is the world's most sophisticated system for characterizing proteins: the LTQ-FT Fourier Transform Mass Spectrometer, coupled to a Two-Dimensional High Performance Liquid Chromatography (HPCL) system, which allows high-performance separation of peptides and their characterization via mass spectrometry. Delivery, installation, start-up, check-out, and operation of this system all occurred at the beginning of the year, as did the relocation of the Windber Research Institute to its new building. Another important aspect of the initial evaluation was an overview of quantitation software for the expressed proteins based on a non-labeling quantitation method.

Trial analyses began with three sample aliquots of patient CV000275 at time points of baseline, 12 weeks, and 1 year, which were digested and sent to the BRIMS facility in Boston, MA for mass spectral and differential expression analysis. A similar analysis was performed in-house on a PF2D protein fractionation system and mass spectrometry analysis was performed via LTQ-FTMS.

The operations and techniques that make up the entire proteomics pipeline for global profiling of blood plasma were developed. Further, in-depth efforts were initiated on the integration and dove-tailing of the various steps and operations. Progress included experimentation in the area of high abundance protein depletions, where the most abundant proteins are depleted, in order to allow the less abundant proteins to be detected, quantitated, and characterized. The available depletion techniques were utilized and optimized for our specific sample sets.

Three digestion protocols were optimized and evaluated on efficiency, completeness, reproducibility, and automation ability. Detailed evaluation via LC/MS and protein identification was conducted to develop initial methods in both liquid chromatography separations (LC) and mass spectrometry detection (MS).

LC methods were developed and optimized for large-population clinical proteomics to maximize protein identifications, reproducibility, sensitivity, detection limits, and robustness. A 2-hour time window for sample injection and analysis on the LTQ-FT will give adequate throughput and keep the cost per analysis lower. Finally, LC performance criteria were evaluated, such as maximized peak width, maximized peak capacity, minimized peak tailing, minimized carry-over, reproducible run times, and reproducible peak heights and areas.

Plasma samples (n=3,564) from peripheral blood have been obtained from participants in Ornish Cohorts #13-20 and four control groups. Thirty sample aliquots from 10 participants (at

time points of baseline, 12-weeks, and 1 year for each participant) are currently undergoing mass spectral analysis and differential expression analysis as a pilot study to assess the performance of the methods outlined above.

Adverse Events

All adverse events are submitted and adjudicated by the Windber Medical Center Institutional Review Board and Ft. Detrick Office of Research after review by both the Principal Investigator and Medical Monitor. There have been 20 adverse events over the course of the study, of which 9 were serious events and 11 were considered non-serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, the 9 events were considered serious due to inpatient hospitalizations and 8 were cardiac related. No deaths occurred and 6 of these adverse events were deemed to be study related and were defined as possible risks in the study consent.

2. Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Previous Subjects in the Dr. Dean Ornish Program for Reversing Heart Disease.

The primary objective of this study is to examine associations between DNA variation (in the form of 500,000+ single nucleotide polymorphisms) and participant response to the program. We are examining the influence of innate genetic variation on overall response, quantified as the risk of future cardiac events (Framingham risk), as well as response of specific cardiovascular disease risk factors. The main hypothesis is that innate variation in genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors (or other genes) will correlate positively with response to intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which may lead to improved CHD risk factor profiles and genetic markers of coronary artery disease reversal or stabilization. Participants in this study are being recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at Windber Medical Center (prior to implementation of the primary Molecular Profiling Protocol described above).

Status: In January 2006 high-density single nucleotide polymorphism assays were initiated on Integrative Cardiac and Metabolic Health Program participants. Initially, ~70 of the Affymetrix 100K chip sets were assayed for 35 participants. Two 50K chips, each containing 50,000 individual SNPs, were run for each participant. Call rates were extremely high, averaging >98% overall. On April 4, 2006, staff at the WRI received training for the Affymetrix 500K SNP analysis. After some minor changes to equipment location and technical procedures, which the Affymetrix technical representative suggested, running of the 500K SNP chips began. Since the training, DNA samples from 10 individuals who participated in the Ornish program have been subjected to two 250K chips containing a total of ~500,000 SNPs. Call rates were very high, averaging ~96%, well above the Affymetrix standard of 93% for the 500K SNP chips. These assays extend the comprehensive genome-wide database for each participant to ~600,000 SNPs. Additional participants will be added to the work flow in the coming year and genome-wide association studies, examining relationships between innate genetic variation and risk factor response will begin.

Task #7: Ongoing enrollment to Acute Endothelial Dependent Responses to Distinct Macronutrient Challenges Study: A Comparison of Brachial Reactivity Responses to a Low Carbohydrate/High Fat, High Carbohydrate/Low Fat, or an AHA Meal in Subjects at Risk for Coronary Heart Disease protocol.

Status: IRB approval was received from the University of Maryland on August 31, 2004; Windber Medical Center on October 29, 2004; and the Uniformed Services University on December 16, 2004. The following panel of biomarkers has been identified: lipid profile (HDL, LDL, total cholesterol), NMR lipid panel, ADMA, hsC-reactive protein, activated factor VII-A, oxidized LDL, PYY, CCK, VCAM-1, adinopectin, ghrelin, leptin, interleukin-6 and-8, tumor necrosis factor, triglycerides, glucose, and insulin.

The primary objective of this study is to evaluate the effect of three varied meals (acute macronutrient challenges) on endothelium-dependent flow-mediated dilation of the brachial artery in subjects with one or two risk factors for coronary artery disease (overweight, hypertension, hyperlipidemia, or a family history of premature coronary artery disease). Secondary objectives include assessing the affect of the macronutrient challenge on various blood biomarkers, including markers of inflammation, coagulation factors, endothelial progenitor cell numbers, and markers of NO function as well as standard lipid, glucose, and insulin levels. Endothelial dysfunction has been implicated as an early event in atherosclerosis and in the pathogenesis of coronary artery, peripheral vascular, and cerebrovascular disease. Impairment of endothelial function has been demonstrated after high glucose and high saturated fat meal challenges in healthy adults. Specifically, we will study the acute effects of a high carbohydrate/low fat (Ornish), low carbohydrate/high fat (Atkins), and standard (American Heart Association) isocaloric, isovolumic meals on endothelial function

Study recruitment and intervention at the University of Maryland is complete. All 24 participants have completed all three meals for the study; all BART studies and blood samples have been collected. Dr. Vogel at the University of Maryland is currently reading the brachial artery reactivity studies and plasma/serum samples are being analyzed at the respective laboratories.

At least three manuscripts (listed below) are in preparation.

1. Brachial artery reactivity in response to three meals of different macronutrient composition
2. Changes in CVD biomarkers in response to three meals of different macronutrient composition
3. Relation of biomarker response to satiety following consumption of three meals of different macronutrient composition

Collaborators for this project are:

Alan Shuldiner, MD – Joslin Center for Diabetes
Robert A. Vogel, MD & Richard B. Horenstein, MD – University of Maryland School of Medicine
Wendy Post, MD – Johns Hopkins Hospital
Christie M. Ballantyne, MD – DeBakey Heart Center, Baylor College of Medicine
Dean Ornish, MD, Preventive Medicine Research Institute

Task #8: Initiate "A Feasibility Study of the Effect of Exercise Intensity on Visceral Fat" Protocol.

Status: Study design/methodology in progress. Not yet submitted to WRAMC IRB for review.

Task #9: Initiate Influence of Exercise and Stress Management on the Metabolic Syndrome protocol.

Status: IRB approval has been received from the Windber Medical Center on Nov 21, 2003 and October 29, 2004. Approval was received from the Uniformed Services University in December 2005. This study will begin in late 2006.

The aims of this study are to characterize in participants diagnosed with metabolic syndrome: 1) longitudinal changes in biochemical variables in blood during an exercise and/or stress management intervention; 2) longitudinal changes in gene and protein expression in blood during an exercise and/or stress management intervention; 3) associations of gene/protein expression profiles and single nucleotide polymorphisms (SNPs) with changes in known risk factors for the metabolic syndrome; and 4) gene/protein expression profiles and SNPs predictive of significant differences among individuals in patterns of change in risk factors for the metabolic syndrome during a six-month exercise and/or stress management intervention.

Key Research Accomplishments

- IRB approval of 2 protocols:
  - *B*etter Adherence *t*o *T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial
  - CADRe Five-Year Follow-up Protocol

- Non-Invasive Coronary Artery Disease Reversal" (CADRe) Study Protocol
  - Final data analysis complete
  - One manuscript published (see Appendix A); 3 manuscripts in preparation

- Dr. Dean Ornish Program for Reversing Heart Disease protocol
  - Manuscript in preparation
  - Subject enrollment to date is 368 participants - 20 cohorts / 4 retreats
  - Age/Gender/Disease state matched control group established to compare risk factor changes

- Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal
  - Subject enrollment in the research protocols totaled 271
  - More than 5,500 biological samples have been collected
  - Comprehensive NMR lipid panel shows significant clinical improvement in Ornish participants – the average LDL-particle number (more strongly linked to CVD risk than LDL levels) improved from a clinically significant category (borderline high) to a non-significant category (near optimal)
  - Participation in the Program may arrest depletion of endothelial progenitor cells (EPCs) – EPCs, which are derived from bone marrow and function in ongoing endothelial repair, decreased significantly in control subjects by the end of the year, but did not change significantly in Ornish participants

- Fundamental molecular changes were shown to occur in Program participants – patterns of global gene expression can differentiate baseline, three month, and one year examinations
- Collection of research results from the first comprehensive cardiac imaging on Program participants
- Protocols developed to begin conducting proteomic studies on Program participants

- Acute Endothelial Dependent Responses to Distinct Macronutrient Challenges Study: A Comparison of Brachial Reactivity Responses to a Low Carbohydrate/High Fat, High Carbohydrate/Low Fat, or an AHA Meal in Subjects at Risk for Coronary Heart Disease protocol enrollment and intervention complete
  - 3 Manuscripts in preparation

Reportable Outcomes

Published Manuscripts/Abstracts:

Marshall, D., Walizer, E., Medendorp , S., Lee, L., & Vernalis, M. The effect of an intensive one-year lifestyle intervention program on carotid intima medial thickness. *(Abstract submitted)*.

Vizza J, Neatrour DM, Felton PM, Ellsworth DL. Effects of cardiovascular lifestyle change on psychosocial risk factors for coronary artery disease. Health Psychol *(in preparation)*

Marshall, D., Walizer, E., Vernalis, M. (2006). Enhancing achievement of healthy lifestyle habits in military healthcare beneficiaries through a lifestyle intervention program. Circulation, 113(8), e301, p. E329.

Marshall, DA., Vernalis, M., Remaley, AT., Walizer, E., Scally, JP., & Taylor, AJ. (2006). The effect of an ultra-low fat diet combined with aerobic exercise on serum lipids and apolipoproteins in an Ornish lifestyle modification program. American Heart Journal, 151, 484-491.

Marshall, D., Walizer, E., Northrup, G., Vernalis, M. (2005). On-site case management is better than telephonic monitoring for weight loss. The Journal of Heart Disease: Abstracts of the 12th World Congress On Heart Disease- New Trends In Research, Diagnosis And Treatment, Vancouver, British Columbia, July 16-19, 4(1): 77.

Marshall, D., Walizer, E., Vernalis, M. (2005). An alternative to high-dose statins for achieving new low density lipoprotein cholesterol goals. Circulation, 111(14), 184-296, p. E220.

Ellsworth, DL., O'Dowd, SC., Salami, B., Hochberg, A., Vernalis, M., Marshall, A., Morris, JA., & Somiari, RI. (2004). Intensive lifestyle modification: Impact on cardiovascular disease risk factors in subjects with and without clinical cardiovascular disease. Preventive Cardiology, 7(4), 168-175.

Kashani, M., Walizer, E., Vernalis, M., Marshall, DA. (2004). Metabolic syndrome patients in an Ornish intensive lifestyle change program exhibit higher perceived stress. Journal of Cardiovascular Nursing, 19(4), 8A.

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Marshall D, Walizer E, Vernalis M. Therapeutic Lifestyle Change (TLC): An Alternative to High-Dose Statins for Achieving New LDL Goals. Army ACP Annual Meeting, Arlington, VA, November 2004. (Oral)

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Conclusions

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could nearly eliminate the development of CHD and substantially decrease CHD morbidity and mortality. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

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Appendix A

Coronary Artery Disease

The role of exercise in modulating the impact of an ultralow-fat diet on serum lipids and apolipoproteins in patients with or at risk for coronary artery disease

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Background Ultralow-fat diets are known to reduce high-density lipoprotein cholesterol (HDL-C) levels. In the setting of a multicomponent lifestyle intervention program, relationships between exercise variables and HDL-C levels were examined to determine whether exercise moderates this dietary effect on serum lipids and apolipoproteins.

Methods We performed a 3-month, prospective, nonrandomized lifestyle intervention study (≤10% dietary fat; aerobic exercise [180 min/wk], group support, and yoga [60 min/d]) in 120 subjects with or at risk for coronary artery disease.

Results After 3 months, dietary fat intake was reduced to 8.7% ± 2.6% of total intake and the median weekly exercise time was 194 minutes. High-density lipoprotein cholesterol levels decreased by 8.3 ± 11.3 mg/dL ($P < .001$), and triglyceride levels increased by 17.6 ± 102.7 mg/dL ($P = .026$). A small dense low-density lipoprotein cholesterol (LDL-C) phenotype emerged indicated by a 13.8% LDL-C reduction accompanied by only a 2.3% reduction in apolipoprotein B levels ($P = .064$). Among subjects with exercise amounts less than those of the group median, HDL-C reductions were greater in those with more than (−13.5 ± 16.0 mg/dL) versus less than (−2.5 ± 7.5 mg/dL) the median reductions in fat intake ($P = .026$). Even among subjects who exercised >194 min/wk, HDL-C was reduced compared with baseline (−7.4 ± 7.9 mg/dL, $P < .001$).

Conclusions An ultralow-fat diet as a component of a comprehensive lifestyle intervention induces reductions in HDL-C and the emergence of a dyslipidemic lipid profile. Aerobic exercise only partially mitigates this effect.
(Am Heart J 2006;151:484-91.)

The scientific basis for recommending dietary fat reduction as an approach to reduce cardiovascular disease dates back to the studies by Keys,[1] which demonstrated that countries with the lowest dietary saturated fat also had the lowest incidence of cardiovascular disease. Subsequently, ultralow-fat diets (≤10% of total caloric intake as fat), emphasizing the amount rather than the type of dietary fat, were promoted in combination with other intensive lifestyle interventions to arrest or reverse coronary artery disease (CAD).[2,3] The Lifestyle Heart Trial,[4,5] including a specific ultralow-fat diet developed by Ornish (the "Ornish Diet"), reported that a higher proportion of patients with CAD following the diet along with an intensive lifestyle intervention showed coronary angiographic improvement in comparison to a usual care control group at both 1-year and 5-year follow-up. These changes in the treatment group were accompanied by improvements in angina pectoris, and total and low-density lipoprotein cholesterol (LDL-C); however, a dyslipidemic profile (lower high-density lipoprotein cholesterol [HDL-C] and increased triglycerides) emerged.

Short-term, controlled dietary studies confirmed that substantially limiting dietary fat intake, while lowering serum LDL-C and total cholesterol, results in unfavorable changes in HDL-C and triglyceride levels.[6-9] Although the net impact on long-term cardiovascular morbidity and mortality of these combined favorable and unfavorable changes in lipid components is unknown, the decrease in HDL-C concentration is of concern based upon evidence that low HDL-C, independent of triglyceride level, is associated with increased coronary heart disease risk.[10] In contrast, exercise has been shown to broadly and favorably impact the lipoprotein profile and is one of the few nonpharmacologic interventions

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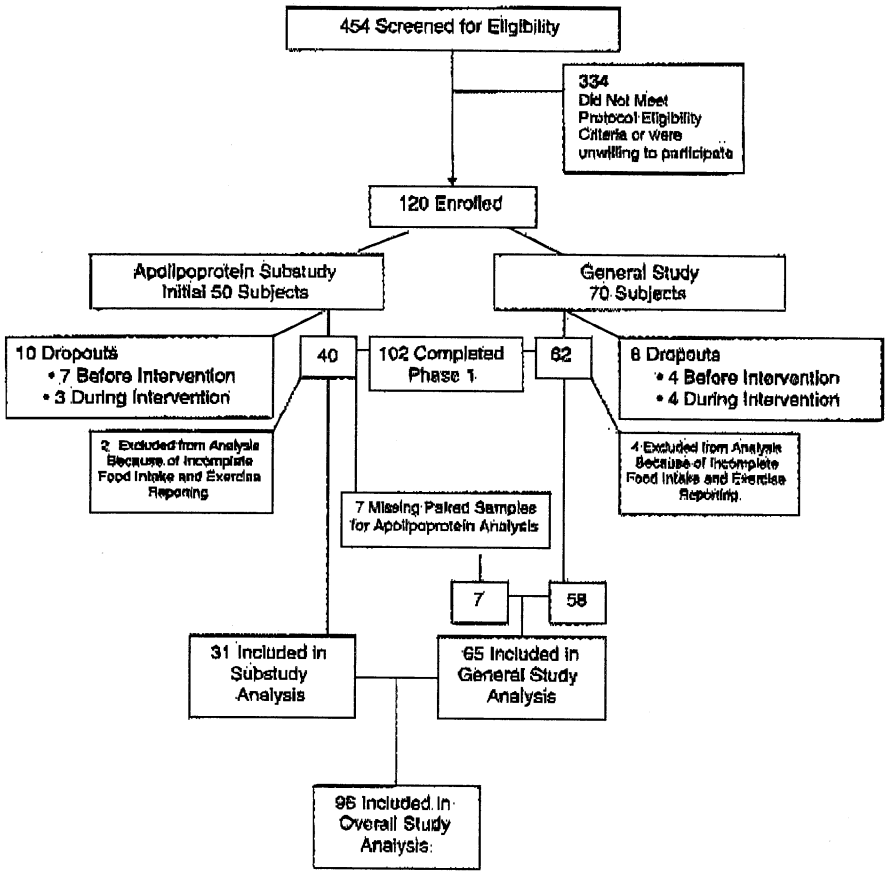
Figure 1



Flow diagram of participants in the trial.

reported to increase HDL-C.[11,12] Thus, exercise could potentially moderate the effect of low-fat diets on the lipid profile.

The purpose of this study was 2-fold. First, we sought to independently investigate the serial changes in serum lipid concentrations specifically those related to HDL-C during the initial 3 months of an Ornish diet and lifestyle program. Second, we sought to examine the relationships between exercise variables and changes in HDL-C levels to determine whether exercise moderates the effect of an ultralow-fat diet on serum lipids and apolipoproteins.

Methods

Study population and design

This is a prospective, single-arm study modeled from the Dean Ornish Program for Reversing Heart Disease[4,5] that was conceived to determine the feasibility and efficacy of this specific lifestyle intervention in a nonresidential military population. The study was funded by a congressional appropriation to the Department of Defense. Volunteer subjects were self-referred, military health care beneficiaries, aged ≥18 years with known coronary risk factors or CAD, willing to make comprehensive lifestyle changes for 1 year. This protocol was approved by the Department of Clinical Investigation/Human Use Committee of Walter Reed Army Medical Center (Washington, DC) and Human Use Committee at the Uniformed Services University for the Health Sciences (Bethesda, MD).

Before eligibility screening of this predominantly sedentary population, which included a complete medical history, physical examination, and treadmill testing, subjects voluntarily provided written informed consent. Exclusion criteria included high-risk treadmill test, unstable CAD/revascularization procedure within 3 months of study enrollment, symptomatic congestive heart failure/ejection fraction of <35%, inability/unwillingness to fully participate in all 4 program components, or substance abuse, including tobacco, within 3 months of study enrollment. Subjects were entered into the study in cohorts of 10 to 20 participants. We screened 454 subjects to enroll in the first 7 study cohorts

(120 subjects) between February 2000 and April 2002. This study pertains to the initial 3 months (phase I) of the study intervention, which was completed by a total of 102 subjects (85%). After exclusion for missing data, 96 subjects were available for analysis. A lower baseline diastolic blood pressure (BP) was the only baseline characteristic that differed between the subjects analyzed and the 24 dropouts. Subjects' flow through the study is shown in Figure 1.

The study begins with a 5-day residential retreat for education and monitoring the initiation of the lifestyle change interventions. During weeks 2 to 12, subjects are on-site twice weekly, 4 hours each visit, for supervised exercise and yoga, group support, and meals with educational lectures. The study intervention consists of ultralow-fat diet (≤10% total calories as fat, 5-10 mg cholesterol/d, soy and legumes as the protein source, limited nonfat dairy products, 35-50 g of fiber, and ≥5 servings of fruit and vegetables daily), aerobic exercise (180 min/wk), group support led by a psychologist, and yoga (poses, deep relaxation, meditation, guided imagery, breathing for 60 min/d).

Data collection and analysis

Outcome variables measured at baseline and 3 months included BP, heart rate, weight, and body mass index (BMI) by a factory-calibrated Tanita Body Composition Analyzer (Model TBF-300A; Tanita, Tokyo, Japan), percentage of body fat (3-site skin fold caliper analysis as described by Pollock et al[13]), fitness (peak metabolic equivalent [MET] level achieved on maximal treadmill exercise tests), and fasting plasma lipids (total cholesterol, LDL-C, HDL-C, triglycerides). Medications were assessed at baseline and any changes in medications or dosage were queried on a weekly basis by study-subject case managers. Total cholesterol, LDL, HDL, and triglyceride values were directly measured on a COBAS INTEGRA analyzer (Roche Diagnostics, Indianapolis, IN). Calculations were made for non–HDL-C (total − HDL-C) and triglyceride-HDL ratio. Consecutive subjects in the initial 2 cohorts had baseline and 3-month sera analyzed for apolipoproteins (apos) A-I, A-II, and B. Paired baseline and 3-month samples were stored at −70°C and simultaneously analyzed using nephrometric analysis on a COBAS Fara II analyzer (Roche) by turbidometric immunoassay. Nutritionist V software (Version 2.2; First DataBank, San Bruno, CA) was used to analyze 3-day food records at baseline and weeks 8 through 12. Daily personal adherence logs were assessed for compliance to the prescribed dietary pattern (weekly percentage of avoidance of meat/poultry/fish or of added oils throughout phase I) and exercise (weekly minutes of structured exercise activity during weeks 8 through 12).

Statistical methods

Subjects who completed the 3-month intervention and who had complete nutrition and exercise compliance data are included in this analysis (n = 96). The apolipoprotein subanalysis included 31 subjects after accounting for dropouts (n = 10), missing paired laboratory samples (n = 7), and incomplete compliance data (n = 2). Changes in outcome variables between baseline and 3 months were evaluated using either a paired *t* test or Wilcoxon signed rank test, as appropriate. Comparison of the intervention effect between groups (statin use vs no statin use or apolipoprotein vs no apolipoprotein analysis) was performed with a *t* test or Mann-Whitney *U* test for independent groups, as appropriate. To explore the influence of exercise and diet on HDL-C changes, a 1-way analysis of variance with adjustment for multiple comparisons was performed on 4 groups determined on the basis of exercise time and decrease from baseline in percentage of fat of total caloric intake for the entire study population: group 1, exercise time and percentage of fat decrease less than median; group 2, exercise time less than median and percentage of fat decrease greater than median; group 3, exercise time greater than median and percentage of fat decrease less than median; and group 4, exercise time and percentage of fat decrease greater than median. Factors associated with the change in HDL-C (dependent variable) were explored in a linear regression model controlling for physiologically relevant independent variables (change in percentage of fat intake, exercise time, MET change, BMI change) along with an interaction term for exercise and percentage of change in fat intake (calculated as the cross product of these 2 variables) and reported as partial correlation coefficients for significant variables. All statistical analyses were performed using SPSS software (Version 12.0; SPSS Inc, Chicago, IL). Values are reported as mean ± SD except where indicated. A 2-sided probability value of ≤.05 was considered statistically significant.

Table I. Baseline characteristics of study participants

Variables	(n = 96)
Male	68
Age (mean ± SD)	61 ± 11 y
Race	
White	83
African American	13
Other	4
Diabetes	17
Hypertension	64
Coronary disease	68
Statin use	71
Employed	44

Values are percentages except where indicated.

Results

Study subjects were predominantly older, white men with documented CAD, hypertension, and statin-treated hyperlipidemia (Table I). The mean subject age was 68 years, 17% had diabetes, 64% were hypertensive, 68% had chronic, stable CAD, and 44% were employed at least part-time. At study entry, >80% of subjects had a BMI of >25 kg/m^2 and their fitness, as measured by peak MET level achieved on maximal treadmill testing (9.2 ± 2.9), was less than average for this age group.[14] As compared with the average US diet, the typical subject's baseline diet was characterized as low-fat (23.3% of total calories) and fiber-enriched (24.1 g/d).

At 3 months, mean weekly exercise frequency was 4.8 ± 0.2 and mean weekly exercise time was

Table II. Major outcome variables at baseline and 3 months

	Baseline	3-m	Change
Nutritional characteristics (n = 82; 14 subjects with missing baseline 3-d food diaries)			
Total intake (kJ/d)	6769 ± 2014	6786 ± 1557	17 ± 2135
Fat (% total intake)	23.3 ± 11.1	8.7 ± 2.6	−14.7 ± 11.0*
Carbohydrate (% total intake)	58.2 ± 12.1	74.4 ± 4.7	16.2 ± 11.8*
Protein (% total intake)	17.6 ± 4.1	15.9 ± 3.1	−1.7 ± 4.6*
Fiber (g/d)	24.1 ± 13.8	43.9 ± 15.8	19.9 ± 3.5*
Body composition and BP (n = 94; 2 subjects missing body fat measures)			
Weight (lb)	190.1 ± 45.3	178.4 ± 40.5	−11.8 ± 9.4*
BMI (kg/m^2)	29.0 ± 5.6	27.2 ± 4.8	−1.8 ± 1.4*
Percentage of body fat (calipers)	28.1 ± 8.3	25.7 ± 7.4	−2.5 ± 2.9*
Systolic BP (mm Hg)	131.4 ± 17.6	123.5 ± 16.2	−7.9 ± 16.8*
Diastolic BP (mm Hg)	73.9 ± 9.4	68.1 ± 8.8	−5.9 ± 11.3*
Standard lipid measurements (n = 96)			
Total cholesterol (mg/dL)	183.3 ± 40.9	161.4 ± 36.6	−21.9 ± 33.9*
LDL-C (mg/dL)	108.2 ± 30.0	91.1 ± 23.6	−17.0 ± 22.0*
HDL-C (mg/dL)	50.6 ± 17.7	42.3 ± 11.0	−8.3 ± 11.3*
Triglycerides (mg/dL)	166.3 ± 95.3	183.9 ± 119.5	17.6 ± 102.7†
Non–HDL-C (mg/dL)	132.7 ± 38.5	119.2 ± 33.8	−13.6 ± 31.5*
Triglyceride-HDL-C ratio	3.8 ± 2.9	4.9 ± 4.7	1.1 ± 3.6*
Total cholesterol–HDL-C ratio	3.9 ± 1.2	4.0 ± 1.1	0.10 ± 0.8‡

Values are presented as mean ± SD.
*$P \leq .001$.
†$P = .026$.
‡$P = .247$.

195.7 ± 80.3 minutes. Fitness improved to at least the 50th percentile MET level for age with an increase of 1.6 ± 1.8 METs (22% increase, $P < .0005$). There was no change in total caloric intake, and subjects reported high dietary compliance, including successful avoidance of meat/fish/poultry 93.3% of the time and successful avoidance of added oils 94.6% of the time. Significant changes were made in dietary macronutrient composition (Table II) with a 14.7% ± 11.0% decrease of total intake as fat, a 16.2% ± 11.8% increase of total intake as carbohydrate, and a 19.9 ± 3.5 g/d increase in fiber ($P \leq .001$ for all values). Body composition and BP demonstrated significant improvement (Table II). Weight and body fat decreased by 5.9% and 8.4%, respectively ($P < .001$). Systolic and diastolic BP decreased by 5.2% and 5.9%, respectively ($P < .001$).

Lipid and apolipoprotein effects

Baseline and 3-month lipid measurements are shown in Table II. At 3 months, significant decreases were observed for total cholesterol (10% decrease), LDL-C (13% decrease), and non–HDL-C (7% decrease). The proportion of subjects with baseline LDL-C of <100 mg/dL increased from 41% to 68% at 3 months ($P < .001$). However, a dyslipidemic pattern emerged with significant 14.5% decrease in HDL-C, 9.2% increase in triglycerides, and significant increase in the triglyceride-HDL ratio (28% increase). High-density lipoprotein cholesterol was reduced in 82% of the subjects who lowered their LDL-C. Lipid measurements stratified by baseline statin use are shown in Table III. There was no significant change in statin use or dose. Baseline cholesterol, LDL-C, and the cholesterol-HDL ratio were lower in the subjects on statin therapy (n = 67) compared with those in subjects not on statin therapy (n = 29); otherwise, there was no significant difference between groups in lipid variables over 3 months. Within each group, changes in lipid outcome variables were significant and comparable to that in the entire study population, except for the cholesterol-HDL ratio and the change in triglycerides that was not significant in the small group not on statins.

A consecutive subgroup of participants (n = 31) had additional serum analysis of apolipoprotein concentrations (Figure 2). Their changes in dietary fat and exercise time were comparable to the overall cohort. In parallel with the decline in HDL-C, apo A-I significantly decreased by 8.0%, whereas apo A-II levels showed a nonsignificant 2.6% decrease ($P = .127$). In contrast to a 13.8% LDL-C reduction, apo B decreased only 2.3% ($P = .064$). These data indicate relative apo A-II enrichment of HDL-C and apo B enrichment of LDL-C.

Interaction of exercise and dietary fat intake on HDL-C

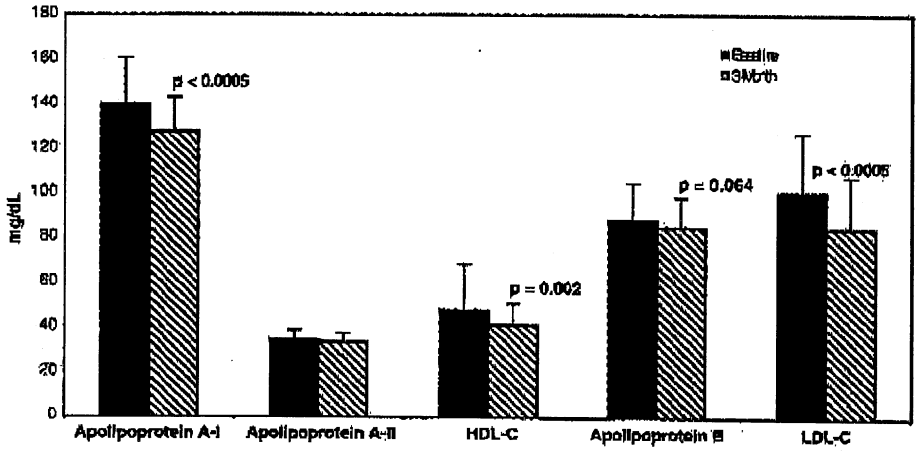
Median exercise time during the study was 194.0 (range 36.0-479.4) min/wk and median change in percentage of fat intake was −13.0% (range −65.0% to 2.0%). Four subsets of subjects were made based upon concurrent changes in these 2 variables: group 1 (exercise time and percentage of fat change less than median); group 2 (exercise time less than median and percentage of fat change greater than median); group 3 (exercise time greater than median and percentage of fat decrease less than median); and group 4 (exercise time and percentage of fat change greater than median). Change in lipid and body composition variables within each of these 4 subsets are shown in Table IV. The subjects who both exercised and changed their diet the least (group 1) demonstrated the smallest HDL-C change (Figure 3). In comparison, the largest HDL-C decrease was observed in group 2 subjects who exercised the least yet reduced their dietary fat intake the most ($P = .026$ vs group 1). In both groups with exercise levels greater than the median (groups 3 and 4), HDL-C decreased significantly from baseline (−7.4 ± 7.9 mg/dL, $P < .001$) and to a similar degree, regardless of the change in dietary fat intake. A linear regression model

Table III. Lipid results stratified by statin use

Variable (mg/dL)	Statin use (n = 67)			No statin use (n = 29)		
	Baseline	3-m	Change	Baseline	3-m	Change
Cholesterol	170.6 ± 35.7	150.4 ± 31.5	−20.2 ± 8.3*	212.7 ± 37.3†	186.8 ± 35.3	−25.9 ± 44.7‡
LDL-C	99.2 ± 25.0	83.7 ± 20.4	−15.4 ± 20.9*	129.0 ± 30.6†	108.2 ± 21.7	−20.8 ± 24.2*
HDL-C	48.8 ± 14.9	41.0 ± 10.5	−7.9 ± 8.8*	54.6 ± 22.8	45.2 ± 11.8	−9.3 ± 15.9*
Triglycerides	171.0 ± 95.0	187.1 ± 111.2	16.1 ± 80.1§	155.6 ± 97.0	176.6 ± 138.5	21.0 ± 143.6
Non–HDL-C	121.8 ± 33.3	109.5 ± 28.6	−12.3 ± 26.5*	158.1 ± 38.1	141.6 ± 34.8	−16.5 ± 41.2‖
Triglyceride-HDL-C ratio	4.1 ± 3.1	5.2 ± 5.1	1.2 ± 3.6*	3.4 ± 2.5	4.2 ± 3.9	0.9 ± 3.6¶
Total cholesterol–HDL-C ratio	3.7 ± 1.1	3.8 ± 1.1	0.1 ± 0.7	4.3 ± 1.3#	4.3 ± 1.2	0.0 ± 1.0

Values are presented as mean ± SD.
*P < .001, baseline versus 3-month.
†P < .001, baseline statin use versus no statin use.
‡P = .004, baseline versus 3-month.
§P = .05, baseline versus 3-month.
‖P = .04, baseline versus 3-month.
¶P = .063, baseline versus 3-month.
#P = .024, baseline statin use versus no statin use.

Figure 2



Baseline and 3-month apolipoprotein, LDL-C, and HDL-C levels in a consecutive subgroup of participants (n = 31).

controlling for changes in percentage of fat intake, BMI, fitness level (MET level), and self-reported exercise time found that the reduction in percentage of fat intake was the only variable with a significant direct relationship to the decrease in HDL-C (r = .27, P = .025).

Sex effects

Men and women had similar demographic characteristics, although there was a trend for a higher percentage of men to be white (87% vs 76%, P = .181), have CAD (73% vs 59%, P = .180), and be employed (53% vs 26%, P = .07). There was no difference in reported exercise time or percentage of MET improvement. However, men had significantly higher MET levels than women at baseline and 3 months. Men had significantly higher body weight than women, but baseline and 3-month changes in BMI were comparable. Women had higher percentage of body fat and greater percentage of body fat loss than men (−3.7 ± 2.7 vs −1.8 ± 2.8, P = .003). Total caloric and fiber intake were greater in men than in women at baseline and 3 months. A trend for greater dietary fat reduction was found for women compared with men (−18.0% ± 9.6% vs −13.1% ± 11.2% of total calories, P = .053).

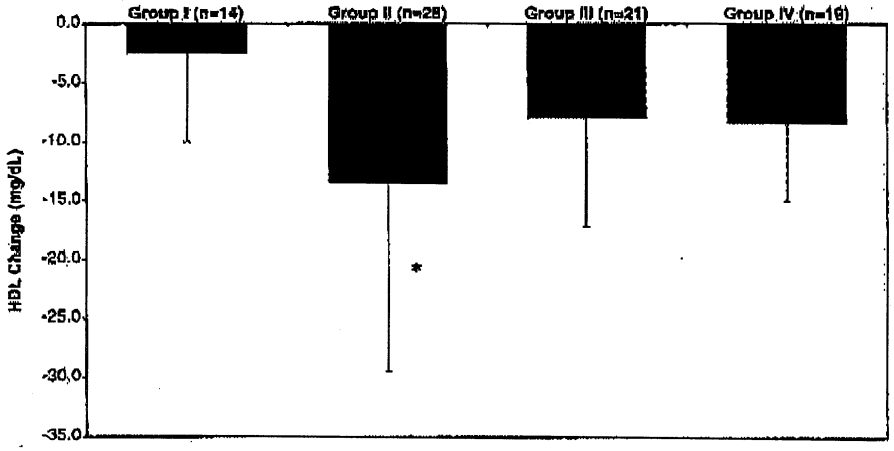
The only lipid parameter demonstrating sex differences was HDL-C, which was significantly higher at baseline and 3 months in women and decreased more in women than men at 3 months (−13.5 ± 15.9 vs −5.5 ± 6.4 mg/dL, P = .006). The influence of exercise and dietary fat reduction on HDL-C levels, as shown in Figure 3, are directionally unchanged with only men in the analysis.

Table IV. Lipid and body composition changes in diet and exercise subgroups

	Group 1 (n = 14)	Group 2 (n = 28)	Group 3 (n = 21)	Group 4 (n = 19)	P (ANOVA)
Exercise time (min)	133.5 ± 14.3	139.0 ± 8.8	248.7 ± 13.8	272.6 ± 16.4	-
Decrease in percentage of dietary fat intake	−6.5 ± 1.2	−22.1 ± 1.6	−5.4 ± 0.9	−20.1 ± 2.7	-
Total cholesterol change (mg/dL)	5.4 ± 12.6	−33.5 ± 5.4	−23.5 ± 6.8	−30.2 ± 5.3	.003
LDL-C change (mg/dL)	−6.0 ± 4.9	−20.0 ± 4.5	−17.9 ± 5.1	−23.8 ± 4.4	.128
HDL-C change (mg/dL)	−2.5 ± 2.0	−13.5 ± 3.0	−8.0 ± 2.0	−8.4 ± 1.5	.035
Triglyceride change (mg/dL)	87.1 ± 48.2	21.9 ± 14.2	3.8 ± 13.6	5.4 ± 21.8	.060
MET change	1.6 ± 0.4	1.5 ± 0.3	2.0 ± 0.5	1.3 ± 0.4	.650
Weight change (lb)	−7.4 ± 1.6	−12.6 ± 1.9	−10.6 ± 1.9	−16.8 ± 2.1	.023
Percentage of body fat change	−1.1 ± 0.6	−3.8 ± 0.5	−2.1 ± 0.7	−2.6 ± 0.4	.019
BMI change (kg/m^2)	−1.1 ± 0.2	−2.0 ± 0.3	−1.6 ± 0.3	−2.5 ± 0.3	.013

Values are presented as mean ± SD unless otherwise noted. *ANOVA*, Analysis of variance.

Figure 3



Influence of exercise and dietary fat reduction on HDL-C levels. Group 1 (exercise time and percentage of fat change less than median), group 2 (exercise time less than median and percentage of fat change greater than median), group 3 (exercise time greater than median and percentage of fat decrease less than median), and group 4 (exercise time and percentage of fat change greater than median). Groups 3 and 4, $P < .001$ compared with baseline. Asterisk, $P = .026$ versus group 1.

An analysis with only women subjects demonstrates a similar pattern, but none of the comparisons are significant because of small numbers in each of the 4 categories.

Discussion

Our findings show that in a population with stable CAD or coronary risk factors, 3 months of participation in this lifestyle modification program resulted in substantial improvements of body composition, BP, and fitness, as well as total and LDL-C. Some of these changes rival what is observed with pharmacologic treatment. Moreover, the improvement in total and LDL-C occurred regardless of whether subjects were taking lipid-lowering drugs. Without a change in caloric intake, improvement in body composition can be attributed to increased exercise. However, despite frequent aerobic exercise in an amount considerably greater than the commonly recommended guideline of 150 min/wk, this ultralow-fat diet was associated with a significant reduction in HDL-C. Furthermore, these changes were associated with adverse changes in the apolipoprotein profile including preferential reduction in apo A-I, and a relative enrichment of LDL-C with apo B consistent with the generation of small dense LDL-C particles. These data, from an independently conducted, Ornish-type lifestyle intervention study, support concerns about the role of ultralow-fat diets in cardiovascular prevention.[6]

Similar findings in standard lipid measurements have been reported from the Ornish Multicenter Lifestyle

Demonstration Project. In 333 patients studied for 3 months, HDL-C decreased 11% and LDL decreased by 14% from baseline values.[4] In the same study, although men had reported significantly more aerobic exercise than women, their HDL-C decline was not significantly different (−11% men, −7% women), which suggests that exercise did not modulate HDL-C levels in that setting. In the original Ornish study of only 23 patients, HDL-C decreased 27% within 1 month of the introduction of an ultralow-fat diet and stress management intervention without exercise.[15] This supports the hypothesis that exercise may otherwise limit what might be a more substantial reduction of HDL-C on an ultralow-fat diet.

Adverse changes in the apolipoprotein pattern are of additional concern. The Lifestyle Heart Trial reported apolipoprotein data in 20 patients, including only apo A-I and apo B measures at noncomparable time points (1 and 5 years) to ours (3 months).[3,4] After 1 year, HDL-C and apo A-I had statistically nonsignificant decreases of 10% and 2%, respectively, whereas LDL-C and apo B significantly decreased by 40% and 23%, respectively. Our study, and a smaller study of Parks et al[16] using the Ornish model, confirm these changes and indicate a shift toward a more atherogenic, small dense LDL-C phenotype. The reductions in HDL-C observed are particularly notable in light of the large amounts of aerobic exercise reported. As our study shows, exercise appears to blunt but not negate the adverse impact of an ultralow-fat diet on HDL-C levels, consistent with the known but modest effect of exercise on HDL-C. A large meta-analysis reported an average 4.6% increase in HDL-C with exercise training.[17] However, translation of this result to a multidimensional lifestyle program requires consideration of the effect on other factors, such as the known negative short-term effect of weight loss on HDL-C levels.[18] Greater weight loss may potentially explain the seemingly paradoxical effect of a higher amount of exercise resulting in a greater decrease in HDL-C (group 3). Further studies including assessments of exercise intensity, a potential modifier of the HDL effect of exercise,[11,12,19,20] and examination of the changes in functionality or efficiency of HDL-C in reverse cholesterol transport would be of interest.

Whether HDL-C reduction would confer an adverse effect on cardiovascular risk in the context of concurrent favorable effects on the lipid profile and other cardiovascular risk variables is unknown. In general, each milligram per deciliter increase in HDL-C concentration is believed to confer a 2% to 3% reduction in cardiovascular risk.[21] Ultralow-fat diet proponents have emphasized that conclusions about increased cardiovascular risk emerge from trials where fat intake is typically in the range of 30% to 40%. However, in the years since these ultralow-fat diets were championed, the lack of sufficient long-term efficacy studies and the potential for metabolic dyslipidemia and nutrient inadequacies mitigate against their recommendation in view of new scientific knowledge about nutrition in the treatment and prevention of cardiovascular disease.

Study limitations

Our study has several limitations including the absence of a randomized control group, small proportion of female subjects, short study duration, inability to evaluate apolipoproteins for the entire study population, no direct measurements of lipoprotein particle size or density, and the use of subject-reported exercise time compliance, without exercise intensity data. The 3-month time interval is sufficient to assess the impact of the low-fat diet on lipid parameters[8]; however, longer term studies may be warranted to determine the effects of sustained cardiovascular fitness with stabilization of weight loss. The question of exercise training intensity and dose would be better substantiated by supervised exercise sessions and/or use of a device that records exercise time and intensity. Also, the complexity of a multicomponent intervention makes it difficult to account for the impact of individual components on outcomes.

Conclusions

An ultralow-fat diet, in the setting of a multicomponent lifestyle intervention that also includes moderately high doses of structured exercise, results in a shift toward a dyslipidemic lipid profile including moderate reductions in HDL-C. Although this effect appears to potentially be mitigated by very high amounts of aerobic exercise, it is not prudent to recommend this diet as part of a lifestyle intervention for coronary risk reduction.

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